JOURNAL OF AGRICULTURAL RESEARCH

VOL. 53 WASHINGTON, D. C., August 15, 1936 No. 4

LETHAL HIGH TEMPERATURES FOR CONIFERS, AND THE COOLING EFFECT OF TRANSPIRATION[1]

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INTRODUCTION

Studies of lethal temperatures for conifers have been confined mostly to experiments on young seedlings, and have been carried out chiefly with the object of determining the temperatures at the soil surface which cause stem lesions or "white spot" injury (*9*).[2] The results show considerable variation, depending upon the methods used and the duration of exposure to heat.

In general, heat injuries most frequently occur to plants exposed to intense insolation. Because leaves are most exposed to direct sunlight they are more commonly injured than other parts once the plant is past the juvenile stage. The purpose of the present study was to determine accurately the lethal high temperatures for the species most widely used in forest planting in the Lake States, not only with the view of obtaining a clue to success or failure in plantations, but also to find the highest safe temperature at which tests of drought resistance might be conducted. It was considered of especial interest to determine whether the cooling effect of transpiration enables plants to withstand a higher temperature at low humidities than in air of high moisture content.

RELATED INVESTIGATIONS

That excessively high temperatures often limit the success of both natural and artificial regeneration of conifer forests has been shown by observations made at the Lake States Forest Experiment Station, and is well attested by the literature. Temperatures of surface soil up to 55° C. have been reported in nurseries by several investigators. The writer has measured a temperature of 53° in the Forest Service nursery at Cass Lake, Minn., and even higher temperatures have been reported from field stations. Toumey and Neethling (*20*) found at Keene, N. H., that during early July maximum temperatures of surface soil in open fields and small openings in the forest were rarely lower than 40°, and in more than 50 percent of the cases observed they were in excess of 50°. On warm days it was not unusual for the soil temperature to remain above 50° for 6 consecutive hours. Similar high temperatures were observed by Li (*12*). On the Huron National Forest in Michigan, surface temperatures in sand of 54° to 72° have been observed. Even at a depth of 1 inch, the soil temperature attained as high a value as 55°. Bates and Roeser (*4*) and Roeser (*17*) report soil temperatures of 67° to 70° on south slopes of the Rocky Mountains in Colorado.

[1] Received for publication Mar. 24, 1936; issued October 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 257.

ON LETHAL HIGH TEMPERATURES

According to Miller (*15*), the actual killing temperature, or thermal death point, for most plant cells has been found to vary between 45° and 55° C.

There is considerable variation among species of plants in the degree of heat that they are able to withstand (*13*). The amount of injury at a given temperature increases with increasing length of exposure, and the higher the temperature, the shorter the exposure required to produce death or a given degree of injury. The thermal death point may be altered by an increased concentration of salts in the cell sap, which raises the coagulation point of colloids.

Clum (*7*), by the use of minute thermopiles which could be inserted in the leaf tissue, found that injury to leaves of lilac (*Syringa vulgaris* L.) and fuchsia (*Fuchsia* sp.) occurred at temperatures of 50° C., while privet (*Ligustrum vulgare* L.) was somewhat more resistant. Arthur and Stewart (*1*) noted severe injury to tobacco (*Nicotiana tabacum* L.) leaves at 53°. Berkley and Berkley (*5*), in an exhaustive study of killing temperatures for cotton plants (*Gossypium* sp.), found the lethal temperature to range from 40° to 84°, depending on the age of the plants and the duration and conditions of exposure.

Heat injuries to North American conifers are not uncommon in nursery beds, where white spot or stem lesions (*9*) may frequently be found at or near the ground surface. Hartley (*9*) found that stem lesions of coniferous seedlings occurred at 52° C. Bates (*3*) studied the resistance of seedlings of lodgepole pine (*Pinus contorta* Dougl.), ponderosa pine (*Pinus ponderosa* Dougl.), Engelmann spruce (*Picea engelmannii* (Parry) Engelm.), and Douglas fir (*Pseudotsuga taxifolia* (Lamb.) Brit.) to excessive temperatures of surface soil when exposed to intense solar and artificial radiation. Accurate control of temperature was not maintained. From these tests he concluded that injury is greatest to the youngest seedlings; seedlings which have survived a definite exposure to excessive heat are not likely to be injured by subsequent exposures of the same severity.

Further studies of the same species by Bates and Roeser (*4*) and by Roeser (*17*) under similar conditions of exposure, as well as of other conifers by Baker (*2*) and by Toumey and Neethling (*20*), confirm the conclusion that resistance to heat increases with increasing age of the plants during the first year of life. Data presented by Bates and Roeser (*4*) indicate quite clearly that the degree of injury increases with increasing time of exposure. Exposures of 30 minutes or longer in air temperature as low as 49° C. caused some injury, while 13 minutes' exposure at 58° caused no injury. Some plants successfully withstood temperatures even up to 83° for a period of 7 minutes.

Toumey and Neethling (*20*) exposed echard pans containing seedlings of Norway pine and white pine 2 to 12 days old to solar radiation through a window, supplemented by radiation from an electric heater. Air temperature within a single test varied from about 36° to 39° C. Heat lesions were caused by soil temperatures of 49.4° to 50.6° when exposure time was as long as 2 hours. Under the conditions prevailing in their test it was impossible to regulate soil temperature accurately, and the results consequently showed deviations.

Baker (*2*) carried out a rather comprehensive series of tests of lethal high temperatures on a number of western conifers. Young seedlings, 1 to 3 months old, with thermocouples inserted in the stem at the

ground line, were exposed in shallow pans to radiation from a radiant electric heater. As soon as definite signs of injury were observed a test was terminated. The actual temperatures involved were determined by thermocouples placed in the air and surface soil. Considerable variation in temperature existed in all tests. The seedlings used were quickly killed when the internal temperature reached 54° C., but temperatures only a few degrees lower were endured for a considerable time. In this respect there was relatively little difference among the species used. A more significant difference among species was the differential between soil temperature and stem temperature, which was as great as 8° to 11° for the coarser stemmed seedlings. This difference between soil temperatures and internal temperatures may explain in large part the inconsistencies reported by other investigators.

Roeser (*17*) tested the heat resistance of coniferous seedlings in another way. Seedlings approximately 58, 71, and 110 days old (calculated from date of sowing) were strung through a board with their roots in water. On this board heated fine sand was sprinkled to a depth of one-fourth inch. A thermometer on the board was used to determine the actual temperature to which the seedlings were exposed. There was considerable difference among the different species used. Fifty-eight-day-old seedlings of ponderosa pine withstood temperatures up to 70° C., Engelmann spruce to 63°, and lodgepole pine only to 59°, while 39-day-old Douglas fir withstood temperatures up to 66°. Variations in killing temperature among different individuals of the same species and age class were as much as 8° to 27°. The most resistant of all, 110-day-old ponderosa pine, withstood temperatures up to 83°.

The only paper reviewed which deals with the effect of high temperatures on conifers beyond the cotyledon stage is that of Korstian and Fetherolf (*10*), who found that 3–1 [3] Engelmann spruce transplants were severely injured and in many cases killed by excessive heat, which killed the cambium at the ground line. These injuries were observed in forest nurseries where the actual temperatures involved could not be determined accurately. The highest temperature measured was 54° C.

ON THE INFLUENCE OF TRANSPIRATION ON THERMAL DEATH POINT

There has always been considerable controversy as to whether transpiration plays an important role in reducing leaf temperature. If it does, plants might be expected to withstand a higher external temperature under conditions which favor rapid transpiration. The comprehensive studies of Clum (*6*, *7*) on the temperature of leaves under various conditions of atmospheric temperature, humidity, and light led him to conclude that transpiration plays only a minor role in reducing the temperature of leaves. For the plants studied he found normal leaves to be only 2° to 4° C. cooler than leaves coated with petrolatum or leaves of plants growing in dry soil. He was unable to establish any relationship between leaf temperature and transpiration rate.

Copeland (*8*) made a determination on the cooling effect of transpiration with leaves of *Ceanothus velutinus* Dougl. and other chaparral species, by measuring the leaf temperatures under conditions

[3] Transplants 3 years in the seedbed and 1 year in the transplant bed.

favoring transpiration and under conditions of minimum transpiration. The technique used was to wrap the leaf around a mercurial thermometer and expose it at normal incidence to the sun's rays. When the upper, stomata-free surface, or the undersurface coated with petrolatum was exposed, the temperature was 8° to 11° C. higher than when the uncoated undersurface was exposed. This difference he attributes mainly to transpiration. Leaves which transpired more slowly showed less difference in temperature under similar conditions of exposure.

Miller and Saunders (*16*) found the temperature of wilted leaves in the sun to be from 1.55° C. (for those of sorghum plants) to 4.65° (for those of cowpeas) higher than that of turgid leaves under similar conditions of exposure. The leaves which showed the greatest difference in temperature between the wilted and turgid conditions also had the greatest difference in transpiration.

By thermodynamic studies on leaves in air, Watson (*21*, *22*) was able to formulate a law of thermal emissivity for leaves. Thermal emissivity, or the giving off of heat by the leaf, includes the heat dissipated by radiation and by conduction to the surrounding air, but not the heat used in the transpiration process. From his own studies and those of Meyer (*14*) on transpiration, Watson concluded that thermal emission is of importance in accounting for the loss of heat from leaves. Since the rate of thermal emission increases rapidly with the temperature differential between the leaf and the surrounding air, he is of the opinion that this factor is the deciding one in preventing the thermal death point being reached under conditions of intense insolation. Watson in his study assumed that leaves obey the theoretical laws of thermodynamics. This is probably so within narrow limits, but under extreme conditions the relative importance of transpiration and thermal emission may be reversed.

Arthur and Stewart (*1*), working with tobacco plants, showed that at high temperatures a coating of petrolatum, such as was used by Watson and others to eliminate transpiration, does not really diminish transpiration to any appreciable extent in tobacco leaves. These workers studied the transpiration rate of tobacco plants when exposed to intense radiation from a 1,000-watt lamp. As the distance between the plants and the lamp decreased, the temperatures of the leaves rose slowly to a maximum of 42° C. With further increase in radiation intensity there was an increase in transpiration but no increase in leaf temperature. However, when a tobacco leaf was enclosed in a tight-fitting cellophane envelope and exposed under similar conditions, the leaf temperature rose within 4 minutes to 53°, and severe injury resulted. From these studies the authors concluded that transpiration of leaves is the factor which makes it possible for them to exist under conditions of high radiation intensity and high temperatures. However, it should be pointed out that the cellophane envelope not only surrounds the leaf with a saturated atmosphere, but also reduces to a considerable extent possibilities of thermal emission to the outside air.

Some observations on the effect of atmospheric humidity on the killing temperatures of conifers were made by Bates and Roeser (*4*), who heated plants in an incubator with approximately saturated air. The temperatures which caused severe injury and death to the plants varied from 61° to 66° C. in the saturated atmosphere, and from 66°

to 83° in normal atmosphere. The lengths of these exposures varied from 1 to 10 minutes in saturated air and from 5 to 14 minutes in normal atmosphere.

Roeser (*17*) found that ponderosa pine and lodgepole pine transpired approximately 13 times more water at 51° C. than at 16°, while for Douglas fir at the same temperatures the increase was 18 times, and for Engelmann spruce, 28 times. There was some difference among species in the promptness with which the increase in transpiration followed an increase in temperature. Generally speaking, the ones which most promptly responded to elevation of temperature with increase in transpiration were more resistant to excessive heat. Roeser's work does not, however, present a clear-cut case for the protective value of transpiration.

The most significant work on the effect of humidity on lethal temperatures is that of Berkley and Berkley on the cotton plant (*5*). These workers took particular pains to control temperature to within ±0.5° C., a much narrower range than was maintained in any of the experiments on conifers described above. They found that the time required to kill all plants varied with temperature. For an exposure of 1 minute the cotton plant was killed at 65° in moist air and 84° in dry air. Throughout the temperature range used there was a difference of at least 5° between the temperature required to produce death in moist air and that required to produce death in dry air. Since excessive heat was applied in the form of air temperature and not in the form of radiation, thermal emission could play no part, hence the excess in temperature required to produce death in dry air may be considered as due in large part to the cooling effect of transpiration. A secondary influence is the greater specific heat of moist air, which may have caused a more rapid heating of the plant tissues.

EXPERIMENTAL TECHNIQUE

The tests undertaken for this study were designed to determine the killing high temperatures for both roots and tops and the influence of variations in relative humidity upon these killing temperatures. No attempt was made to simulate field conditions. Exposures were made in a water bath, in moist air, and in dry air.

The four conifers most commonly employed in forest planting in the Lake States region were used in the tests: Norway pine (*Pinus resinosa* Solander), white pine (*Pinus strobus* L.), jack pine (*Pinus banksiana* Lamb.), and white spruce (*Picea glauca* (Moench) Voss). The plants used were from stock which had been growing in a greenhouse for at least 2 years. All stock less than 3 years of age was raised from seed in the greenhouse. During the summer months temperatures in the greenhouse were usually higher than outside air temperatures, so all plants used had probably survived exposure to air temperatures as high as 45° C.

THE WATER BATH

The plants were lifted from the greenhouse bench and sorted as nearly as possible into lots of equal health and vigor. Five plants of each species and age class were provided for each of the following three types of exposure: Roots only in water; tops only in water; plants completely submerged. The roots of plants exposed with tops only in water were packed in damp sphagnum moss to prevent drying.

TABLE 1.—*Temperatures and periods of exposure used in submergence tests of young conifers of different age classes*

2-HOUR EXPOSURES

Species and age class	Temperature [1]												
	52.8° C.	51.7° C.	51.3° C.	50.3 °C.	48.9° C.	48.6° C.	48.4° C.	48.2° C.	47.6° C.	47.1° C.	46.9° C.	45.9° C.	45.7° C.	44.3° C.
Norway pine:														
1–0								x		x	x		x	
1–1								x					x	
1–2	x	x	x	x	x	x			x	x	x			
White pine:														
1–0								x		x	x		x	
1–1								x					x	
1–2	x	x	x	x	x	x			x	x	x			
Jack pine: 1–0		x			x	x		x	x	x	x		x	
White spruce: 1–0							x					x		x

5-HOUR EXPOSURES

Norway pine:														
1–0							x					x		x
1–1							x					x		x
White pine:														
1–0							x					x		x
1–1							x					x		x
Jack pine: 1–0							x					x		x
White spruce: 1–0							x					x		x

[1] "x" indicates that plants of the species and age class shown at the left were exposed at the temperature indicated.

In order to be assured that no injury would result from submergence in water as such, four tests of 2 hours duration and one of 5 hours duration were run with the plants exposed in similar manner in water at room temperature. All species and age classes were used in these tests, and in no case was any injury observed.

Following exposure, the plants were returned to the greenhouse and heeled in. After sufficient time had elapsed for injuries to be readily discernible, usually a week or more, each plant was dug up and carefully examined for injury to fibrous roots, main roots, stem, needles, and buds. Control plants were examined in the same manner.

The water bath used consisted of a double-walled, cork-insulated copper vessel, equipped with an electric stirrer and electric immersion heater, controlled by a sensitive mercury thermoregulator through a relay.[4]

The species of plants, age classes, and temperature conditions in the various tests are shown in table 1. Temperatures were read at 10- to 20-minute intervals for the 2-hour exposures and at 15- to 30-minute intervals for the 5-hour exposures. Variations in temperature were not more than 0.2° except for a slight drop when the plants were first introduced. Any significant deviations were corrected as soon as detected by adding hot or cold water.

EXPOSURE TO MOIST HEAT

For determining the killing temperatures for conifers in hot air of high relative humidity the apparatus developed by the writer for testing drought resistance was used (*19*). This so-called "drought machine" (fig. 1) consists of a cylindrical chamber 30 inches in diameter provided with electric lamps, a thermostatically controlled electric heating coil, and a revolving table for the plants. Air is forced through a "tower" of calcium chloride into the chamber. To obtain high relative humidity, the calcium chloride was replaced by a fine spray of hot water that brought the humidity up to 80 to 94 percent. Under these conditions droplets of water usually collected on the test plants. The radiation intensity from lamps alone at the average heights of the plants was measured with the Shirley radiometer (*18*). Illumination was measured with the Macbeth illuminometer. Wet- and dry-bulb thermometers were read at 15- to 30-minute intervals during the course of each test, and at the end of each test the soil temperature was determined from a thermometer thrust into the soil. For practically all tests temperatures were maintained within $\pm 1.5°$ C., except for rather considerable drops, when the plants were first placed in the machine.

The conditions prevailing during the various tests, together with the species, number, and age of plants used are given in table 2. The temperature differential between tests was reduced near the critical value. Soil temperatures on 5-hour runs averaged about 2° C. below air temperatures. Relative humidities, except for the first two tests, averaged about 90 percent. With a few exceptions two radiation intensities prevailed—0.093 and 0.131 gram calories

[4] The writer is indebted to Dr. R. B. Harvey, of the Department of Plant Pathology and Botany, University of Minnesota, for the use of the necessary laboratory equipment for conducting the submergence tests.

per square centimeter per minute. Three degrees of illumination were used—7.6, 17.1, and 28 footcandles.

The test plants had been grown in the greenhouse under the same conditions as those used in the submergence experiments, the 1–2

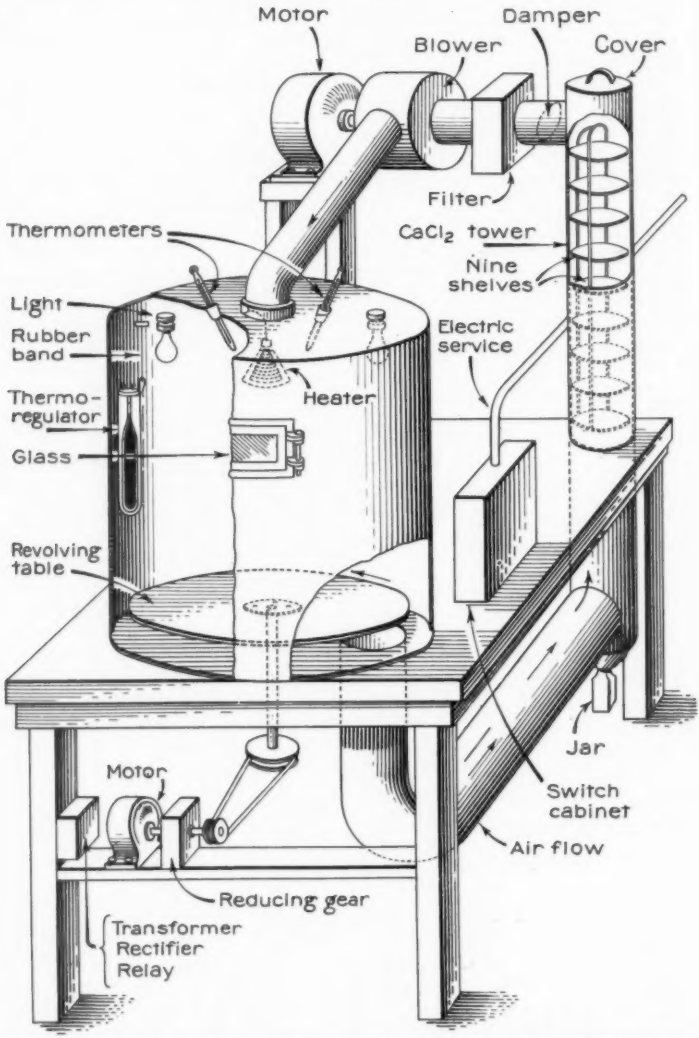

FIGURE 1.—The "drought machine." The trays were removed from the calcium chloride tower and replaced by a spray jet which provided a fine mist of warm water for producing high relative humidity.

Norway and white pines being from the same lot of plants. The plants tested were transplanted in a light sandy soil in 4-inch clay flowerpots or tin cans. All transplanting was done at least 2 weeks

TABLE 2.—*Conditions during tests of young conifers of different age classes in hot air of high relative humidity*

Test no.	Number and age class of species tested								Air temperature			Soil temperature	Relative humidity			Radiation intensity	Illumination
	Norway pine		White pine		Jack pine		White spruce		Mean	Maximum	Minimum		Mean	Maximum	Minimum		
	Number	*Age class*	*Number*	*Age class*	*Number*	*Age class*	*Number*	*Age class*	*°C.*	*°C.*	*°C.*	*°C.*	*Percent*	*Percent*	*Percent*	*Gram-calories per square centimeter per minute*	*Foot-candles*
2-HOUR EXPOSURES																	
4	10	1-2	9	1-2					54.0	55.0	53.0	50.5	86.7	95.0	79.0	0.131	28.0
5	15	1-2	14	1-2					50.2	51.0	49.0	49.0	87.2	95.0	75.0	.131	28.0
6	9	1-2	9	1-2					58.1	59.0	57.0	55.0	89.2	95.0	81.0	.131	28.0
7	13	1-2	15	1-2					52.0	52.7	51.5	49.0	91.9	97.0	75.0	.131	28.0
12	5	1-2	5	2-1-1	5	1-2	5	1-2	45.8	49.0	44.0		89.3	95.0	74.0	[1].093	17.1
5-HOUR EXPOSURES																	
1	10	1-2	9	1-2					51.6	53.0	51.0		65.2	80.0	62.0	0.131	28.0
2	9	1-2	10	1-2					58.8	60.5	55.0		81.9	95.0	75.0	.131	28.0
3	18	1-2	17	1-2					49.6	51.0	48.0		89.9	97.5	84.0	.131	28.0
8	21	1-2	13	1-2					49.9	50.0	49.5	48.0	94.5	95.0	89.0	.064	7.6
9	5	2-1-1	5	2-1-1	5	1-2	5	1-2	52.6	53.2	52.0	50.0	91.6	99.0	85.0	.063	7.6
10	5	2-1-1	5	2-1-1	5	1-2	5	1-2	52.1	52.5	51.5	50.0	92.7	97.0	85.0	.093	17.1
11					5	1-2	5	1-2	51.4	52.0	50.0		89.2	95.0	85.0	.093	17.1
13	5	1-2	5	2-1-1			5	1-2	48.1	50.0	46.0	46.5	90.5	100.0	74.0	.093	17.1
14	5	1-2	5	2-1-1	5	1-2	5	1-2	48.0	49.0	47.0	46.2	88.4	92.0	79.0	.093	17.1
15	5	1-2	5	2-1-1	5	1-2	5	1-2	47.9	50.0	47.5	46.0	90.4	94.0	84.0	.093	17.1
16	5	1-2	5	2-1-1	5	1-1	5	1-1	47.8	48.0	47.5	44.5	85.0	95.0	74.0	.093	17.1
17	5	1-2	5	2-1-1	5	1-1	5	1-1	48.9	50.0	48.0	47.0	89.7	95.0	70.0	.093	17.1
16-HOUR EXPOSURES																	
18	5	1-2	5	2-1-1	5	1-1	5	1-1	37.0	38.0	36.0	33.0	97.0	100.0	94.0	0.032	17.1

[1] The heating unit functioned for 20 minutes only

prior to testing. The plants were removed from the greenhouse and thoroughly watered before being placed in the testing machine. At the end of the tests, the plants were returned to the greenhouse and watered again. A week or more later, the needles, buds, and stems were examined carefully to determine injury, if any. Roots of a few plants were examined but showed no injury.

To make certain that humidity alone or the testing procedure did not influence the results, one test of 16 hours' duration was run with all four species at 37° C. with the relative humidity averaging 97 percent. Under these conditions not a single plant was injured.

EXPOSURE TO DRY HEAT

For tests in hot, dry air the drought machine (fig. 1) was operated in the regular manner. The plants were placed on the revolving table and illuminated with incandescent lamps. The air in the chamber was circulated through the calcium chloride tower two to three times per minute. Temperatures were maintained with the thermostatically controlled electric heater. Illumination was varied during these tests from darkness, i. e., radiation from electric heater only, mostly infrared (radiometer reading 0.12 gram-calories per square centimeter per minute, illuminometer reading 0.02 footcandles), to the light from two 300-watt clear incandescent lamps (radiometer reading 0.45 gram-calories per square centimeter per minute, illuminometer reading 306 footcandles).

The conditions prevailing during the tests and the types of plant used are given in table 3. Temperatures were held to within $\pm 1°$ C. For most temperatures two to three tests were run. Results of tests that for any reason were not properly controlled were discarded in determining critical temperatures. Soil temperatures were from 5° to 20° lower than air temperatures in these tests.

Because of evaporation from the soil it was not practicable to reduce the humidity below about 15 percent. For 5-hour tests, although the maximum values were the same, the averages were much lower. The low humidities correspond with the average minimum values occurring in the field on hot, dry summer days in the Lake States region.

The plants were from the same lot as those used in preceding tests. The soil moisture in the pots was adequate but below the water-holding capacity, since with saturated soil it was impossible to keep the humidity down. Samples taken after each run showed that in almost every case there was at least 5 percent moisture in the soil, which is well above the wilting coefficient for the sandy soil used. Similar plants live from 1 to 3 weeks in the same soil at 2 percent moisture when exposed to continual drying in the drought machine.

After the run was completed the plants were returned to the greenhouse and left there a week or more before final examination.

TABLE 3.—*Conditions during tests of young conifers of different age classes in hot air of low relative humidity*

Test no.	Number and age class of species tested								Air temperature			Soil temperature	Relative humidity			Radiation intensity	Illumination
	Norway pine		White pine		Jack pine		White spruce		Mean	Maximum	Minimum		Mean	Maximum	Minimum		
	Number	Age class	Number	Age class	Number	Age class	Number	Age class	°C.	°C.	°C.	°C.	Percent	Percent	Percent	Gram-calories per square centimeter per minute	Foot-candles
2-HOUR EXPOSURES																	
23	10	2-1-1	10	2-1-1					57.7	58.2	57.5	41.5	20.2	24.0	13.0	0.126	49.0
24	10	2-1-1	10	2-1-1					54.8	54.0	55.0	42.0	23.4	27.0	17.0	.126	49.0
29	5	2-1-1	10	2-1-1	5	1-1	5	1-1	54.3	55.0	53.0	35.0	22.1	25.0	18.0	.126	49.0
21	9	2-1-1	11	2-1-1					54.0	54.5	53.0		23.2	31.0	18.5	.121	93.9
28	5	1-2	5	2-1-1	5	1-1	5	1-1	53.7	54.0	52.0	35.0	22.5	24.0	19.0	.121	49.0
36	4	* 2-1-1	4	1-1	4	1-1	4	1-1	53.3	54.0	52.5	34.0	17.3	21.0	12.0	.451	306.0
35	4	2-1-1	4	1-1	4	1-1	4	1-1	53.2	53.2	52.5	38.5	16.8	21.4	11.0	(1)	(1)
34	4	2-1-1	4	1-1	4	1-1	4	1-1	52.7	53.5	51.5	39.0	13.1	16.3	9.0	(1)	(1)
22	10	2-1-1	10	2-1-1					52.5	54.0	52.0		21.2	26.0	14.0	.121	93.9
20	10	2-1-1	10	2-1-1					52.5	54.0	48.0	35.0	23.5	26.0	21.0	.121	93.9
25	5	2-1-1	5	2-1-1	5	1-1	5	1-1	52.3	53.0	52.0	32.5	22.5	28.0	12.0	.126	49.0
27	5	1-2	5	2-1-1	5	1-1	5	1-1	51.5	52.5	50.0	33.5	25.1	31.0	23.0	.126	49.0
26	5	2-1-1	5	2-1-1	5	1-1	5	1-1	50.5	52.5	51.5	32.0	24.7	26.5	21.5	.126	49.0
19	11	2-1-1	9	2-1-1					48.9	49.0	48.5	30.7	21.2	27.0	17.0	.121	93.9
5-HOUR EXPOSURES																	
47	4	2-1-1	4	2-1-1	2	1-1	4	1-1	56.5	57.0	55.5	43.5	18.4	21.5	16.0	0.451	306.0
52	4	2-1-1	4	2-1-1	2	1-0	4	1-1	55.9	56.0	55.0		20.6	23.0	19.0	.451	306.0
51	4	2-1-1	4	1-1	2	1-0	4	1-1	55.8	56.0	54.0	40.0	19.9	23.0	15.0	.451	306.0
45	4	2-1-1	4	2-1-1	4	1-1	4	1-1	55.0	55.5	54.5	44.2	17.2	23.0	14.0	.451	306.0
37	4	1-1	4	2-1-1	4	1-1	4	1-1	54.8	57.0	53.0	37.0	17.1	22.0	13.0	.451	306.0
44	4	2-1-1	4	2-1-1	4	1-1	4	1-1	54.2	54.5	52.5	49.0	18.3	20.5	17.0	.451	306.0
33	4	2-1-1	4	2-1-1	4	1-1	4	1-1	54.0	54.0	54.0	39.0	15.0	18.0	13.0	.451	306.0
50	4	2-1-1	4	2-1-1	4	1-0	4	1-1	53.9	54.0	53.0	46.4	18.7	22.5	13.0	.451	306.0
49	4	2-1-1	4	2-1-1	4	1-0	4	1-1	53.0	53.5	52.3	46.0	16.4	19.7	15.0	.451	306.0
39	4	2-1-1	4	2-1-1	4	1-1	4	1-1	52.8	53.0	52.0	42.5	16.1	19.0	15.0	.451	306.0
42	4	1-1	4	2-1-1	4	1-1	4	1-1	52.5	52.5	52.0		20.0	24.5	17.5	.451	306.0
40	4	2-1-1	4	2-1-1	4	1-1	4	1-1	52.0	52.0	52.0	43.1	19.6	23.5	16.0	.451	306.0
32	5	1-1	5	1-1	5	1-1	5	1-1	52.0	53.0	51.5	43.0	18.7	22.7	12.0	.451	306.0
48	4	2-1-1	4	2-1-1	4	1-1	4	1-1	51.9	53.0	50.0	45.4	15.8	19.0	14.0	.451	306.0
43	4	1-1	4	2-1-1	4	1-1	4	1-1	51.8	52.5	50.5	46.5	20.4	25.5	17.5	.451	306.0
41	4	2-1-1	4	2-1-1	4	1-1	4	1-1	51.0	51.5	50.5	45.7	19.4	22.5	16.9	.451	306.0
31	10	2-1-1	10	2-1-1					50.0	51.0	49.0	36.0	18.8	21.0	17.0	.451	306.0

[1] Dark.

EXPERIMENTAL DATA

THE WATER BATH

Submerging the tops of plants in hot water resulted in no injury to roots, nor did submergence of roots result in injury to tops. However, after a week or more, plants which had either top or root killed by the exposure died completely. The length of the period during which one part was able to survive with the other dead was greater in older plants.

TABLE 4.—*Results of exposing tops, roots, or entire young conifers of different age classes to hot water for various lengths of time*

LOWEST KILLING TEMPERATURE [1]

Length of exposure and parts submerged	Part of plant	Norway pine			White pine			Jack pine	White spruce
		1-0	1-1	1-2	1-0	1-1	1-2	1-0	1-0
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
2 hours:									
Tops only	Buds	(48.2)	(48.2)	48.9	(48.2)	(48.2)	48.9	48.2	(48.4)
	Needles	(48.2)	(48.2)	51.3	(48.2)	(48.2)	51.3	48.2	(48.4)
	Stems	(48.2)	(48.2)	51.3	(48.2)	(48.2)	51.3	48.2	(48.4)
Roots only	Roots	*45.7*	48.2	50.3	*45.7*	48.2	50.3	*45.7*	*44.3*
Entire plant	Buds	46.9	48.2	50.3	46.9	48.2	50.3	*45.7*	45.9
	Needles	46.9	48.2	50.3	46.9	48.2	50.3	*45.7*	45.9
	Stems	46.9	48.2	51.7	46.9	48.2	51.3	*45.7*	45.9
	Roots	46.9	*45.7*	50.3	*45.7*	*45.7*	50.3	*45.7*	*44.3*
5 hours:									
Tops only	Buds	48.4	48.4	.....	(48.4)	48.4	.....	45.9	(48.4)
	Needles	48.4	48.4	.....	(48.4)	48.4	.....	45.9	(48.4)
	Stems	48.4	(48.4)	.....	(48.4)	(48.4)	.....	45.9	(48.4)
Roots only	Roots	45.9	*44.3*	.....	*44.3*	45.9	.....	*44.3*	*44.3*
Entire plant	Buds	*44.3*	45.9	.....	*44.3*	45.9	.....	*44.3*	*44.3*
	Needles	*44.3*	45.9	.....	*44.3*	45.9	.....	*44.3*	*44.3*
	Stems	*44.3*	45.9	.....	*44.3*	(48.4)	.....	*44.3*	*44.3*
	Roots	*44.3*	45.9	.....	*44.3*	45.9	.....	*44.3*	*44.3*

LOWEST TEMPERATURE CAUSING INJURY [2]

2 hours:									
Tops only	Buds	47.1	(48.2)	47.6	(48.2)	(48.2)	*46.9*	47.1	48.4
	Needles	46.9	*45.7*	47.1	(48.2)	48.2	*46.9*	46.9	48.4
	Stems	47.1	(48.2)	48.9	(48.2)	(48.2)	48.9	47.1	48.4
Roots only	Roots	*45.7*	*45.7*	*46.9*	*45.7*	*45.7*	*46.9*	*45.7*	*44.3*
Entire plant	Buds	*45.7*	*45.7*	*46.9*	46.9	*45.7*	47.1	*45.7*	*44.3*
	Needles	*45.7*	*45.7*	*46.9*	46.9	*45.7*	47.1	*45.7*	*44.3*
	Stems	*45.7*	45.7	*46.9*	46.9	48.2	47.6	*45.7*	*44.3*
	Roots	*45.7*	*45.7*	*46.9*	*45.7*	*45.7*	*46.9*	*45.7*	*44.3*
5 hours:									
Tops only	Buds	*44.3*	*44.3*	.....	48.4	45.9	.....	*44.3*	48.4
	Needles	*44.3*	*44.3*	.....	48.4	44.3	.....	*44.3*	48.4
	Stems	*44.3*	*48.4*	.....	48.4	48.4	.....	*44.3*	48.4
Roots only	Roots	*44.3*	*44.3*	.....	*44.3*	*44.3*	.....	*44.3*	*44.3*
Entire plant	Buds	*44.3*	*44.3*	.....	*44.3*	*44.3*	.....	*44.3*	*44.3*
	Needles	*44.3*	*44.3*	.....	*44.3*	*44.3*	.....	*44.3*	*44.3*
	Stems	*44.3*	*45.9*	.....	*44.3*	45.9	.....	*44.3*	*44.3*
	Roots	*44.3*	*44.3*	.....	*44.3*	*44.3*	.....	*44.3*	*44.3*

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used, and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used, and therefore may not be the lowest that would cause injury.

Complete data on all tests were summarized in large tables with symbols designating the degree of injury. These tables were used to analyze the results, and the conclusions presented were drawn directly from them. The tables presented in the text are made up of the more important values extracted from these general tables. While the

major points are clearly illustrated in the tabular material presented, unfortunately some of the minor points are less distinct than in the complete data.

The critical killing temperatures, as determined by these tests, are given in table 4. The values may be referred to table 1 to determine the highest temperature which did not kill. For instance, the killing temperature with 2-hour top exposure for needles of 1–2 Norway pine, is, from table 4, 51.3° C. The next lower temperature used (table 1) was 50.3°, which failed to kill the needles completely.

An examination of table 4 reveals certain fairly definite trends regarding the resistance of different species and age classes to high temperatures. As would be expected, 5-hour exposures caused killing at lower temperatures than 2-hour exposures.

Between Norway and white pine there appears to be practically no difference in killing temperature. Spruce, when completely submerged, was killed at a temperature about 1° C. lower than Norway and white pine of the same age, while jack pine was the most tender of all. In resisting injuries due to high temperature, both white pine and white spruce appear to have a slight advantage over Norway pine and jack pine, whether the exposure be of 2-hour or 5-hour duration. The older age classes were able to survive higher temperatures than the younger ones quite irrespective of exposure time. However, greater age did not change susceptibility to injury. In every case the first parts to show injury were the fibrous roots, younger needles, and tender new stems. Injury was related to age of tissue or organ rather than to total age of plant. This was particularly evident when the plants were examined. If completely submerged, the tender fibrous roots were killed first, followed by the younger needles and new stem growth. Further increases in temperature injured the older needles and heavier roots, and finally the stem. The portion above the root collar, which is most massive and best protected by insulating bark, was with all plants the last to be killed.

Complete submergence resulted in injury and death to tops at lower temperatures than when only the tops were exposed to the hot water. In only 1 case out of 24 did a temperature as low as 45.7° C. for 2 hours cause important injury to tops when only this part was exposed, but when the entire plant was exposed to this temperature injury occurred in 14 cases. This may have been due to the greater physiological shock produced by killing of large amounts of tissue. It has been shown that dead root systems may supply the top of a plant with water for several days after death (*11*). Undoubtedly toxic substances from decaying roots would be swept along in the transpiration stream, adding to the injury already caused by heat.

EXPOSURE TO MOIST HEAT

The average killing temperature for all species exposed for 5 hours to hot air of high relative humidity was about 51° C. (table 5). These tests revealed no significant differences among either species or age classes. The needles were killed at lower temperatures than the buds and stems, and new growth was killed at lower temperatures than old growth.

TABLE 5.—*Effects of high temperatures in moist air on young conifers of different age classes*

Length of exposure and plant part	Norway pine		White pine		Jack pine		White spruce	
	2-1-1	1-2	2-1-1	1-2	1-2	1-1	1-2	1-1
LOWEST KILLING TEMPERATURE[1]								
2 hours:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Buds	.....	58.1	.....	58.1	.....	.....	.....	.....
Needles	.....	52.0	.....	52.0	.....	.....	.....	.....
Stems	.....	58.1	.....	58.1	.....	.....	.....	.....
5 hours:								
Buds	*51.4*	51.6	52.1	51.6	51.4	(48.9)	51.4	(48.9)
Needles	*51.4*	49.9	51.4	49.6	51.4	(48.9)	51.4	(48.9)
Stems	*51.4*	51.6	52.6	51.6	52.1	48.9	51.4	(48.9)
LOWEST TEMPERATURE CAUSING INJURY[2]								
2 hours:								
Buds	.....	50.2	.....	52.0	.....	.....	(45.6)	.....
Needles	.....	50.2	.....	50.2	.....	.....	(45.6)	.....
Stems	.....	50.2	.....	52.0	.....	.....	(45.6)	.....
5 hours:								
Buds	.....	47.8	51.4	.....	*47.9*	(48.9)	*47.9*	*47.8*
Needles	.....	*47.8*	47.8	.....	*47.9*	*47.8*	*47.9*	*47.8*
Stems	.....	48.9	51.4	.....	*47.9*	(48.9)	48.1	(48.9)

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause injury.

The killing temperature in moist air with 5 hours' exposure seems to be somewhat lower than the killing temperature in water with 2 hours' exposure or less.

Temperatures causing injuries to the plants are also shown in table 5. The differences among species and age classes are greater in regard to injurious temperatures than to lethal temperatures. This was undoubtedly due in part to the uneven stages in vegetative development of the plants tested. An old plant with tender needles will show injury at about the same temperature as a newly germinated seedling but death of the entire plant does not occur until much higher values are attained. Again, the stem and bud were usually more resistant than the needles. The younger age classes appear to have the advantage in their resistance to injury at high temperatures. This is probably due in large part to the greater height of older plants and the consequently greater radiation intensity to which they were exposed. Spruce was least resistant to injury and white pine most resistant. Jack pine appears to be decidedly more resistant than Norway pine.

Resistance to injury at high temperatures is not correlated with recovery from injury. It was found that in this respect Norway pine is the poorest of the lot and white pine and white spruce the best. When Norway pine has all its needles and buds killed, the plant usually succumbs even though the stem may be little injured. White pine and white spruce, on the other hand, will send out new buds from the heavier portion of the stem and eventually recover. Jack pine more closely resembles Norway pine in its inability to recover from injuries due to high temperatures.

EXPOSURE TO DRY HEAT

The significant results of the tests of effect on conifers of air of high temperature and low relative humidity are given in table 6. The killing temperature for 2-hour exposures in dry air for needles of the larger plants was 54° and for stems 57.7° C. For 5-hour exposures the killing temperatures varied from 52° C. for needles to over 56.5° for stems. These values are significantly higher than the corresponding ones for the tests in moist air. The lowest temperatures causing injury were also about 3° to 5° higher than those in moist air. Again the stems were most resistant to high temperatures and the needles least so. Except for the 1–0 jack pine, which was particularly tender, there seems to be little difference due to age class, and this difference is probably due more to the stage of vegetative development, to the greater radiation intensities endured by the taller plants, and to the greater drought stress of larger plants, than to any inherent changes in resistance with age beyond the juvenile stages.

The differences due to species were also slight, but because of their preponderance in one direction they appear to be important. During the examination the species were listed in the order of greatest injury. Out of 30 tests of Norway pine and white pine, the former received the greater injury 24 times. There was no correlation between age class and susceptibility of Norway pine to greatest injury.

TABLE 6.—*Effects of high temperatures in dry air on young conifers of different age classes*

LOWEST KILLING TEMPERATURE [1]

Length of exposure and plant part	Norway pine			White pine		Jack pine		White spruce
	2-1-1	1-2	1-1	2-1-1	1-1	1-1	1-0	1-1
2 hours:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Buds	54.8	(53.7)	.....	54.8	.....	(54.3)	.....	(54.3)
Needles	54.0	(53.7)	.....	54.8	.....	(54.3)	.....	(54.3)
Stems	57.7	(53.7)	.....	57.7	.....	(54.3)	.....	(54.3)
5 hours:								
Buds	56.5	.....	54.8	54.8	(55.8)	54.8	*53.0*	54.8
Needles	53.9	.....	52.0	54.8	55.8	54.8	*53.0*	54.8
Stems	(56.5)	.....	(54.8)	(56.5)	(55.8)	(56.5)	*53.0*	(56.5)

LOWEST TEMPERATURE CAUSING INJURY [2]

2 hours:								
Buds	50.2	53.7	.....	53.7	53.3	52.7	.....	52.7
Needles	*48.9*	*51.5*	.....	*48.9*	52.7	*50.2*	.....	*50.2*
Stems	54.8	53.7	.....	54.8	(53.3)	(54.3)	.....	(54.3)
5 hours:								
Buds	52.0	.....	52.0	52.0	55.8	*51.0*	.....	52.0
Needles	*50.0*	.....	*51.8*	51.0	*52.0*	*51.0*	.....	*50.0*
Stems	53.9	.....	54.8	53.9	55.8	54.0	.....	54.0

[1] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause death. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause death.

[2] Figures in parentheses represent the highest temperatures used in the experiment, which in this case did not cause injury. Figures in italics are lowest temperatures used and therefore may not be the lowest that would cause injury.

All four species were represented in 24 test runs. In these, Norway pine received greatest injury 15 times, white pine 4 times, jack pine 3 times, and white spruce twice. Rating the species according to

relative susceptibility to injury, with the highest score representing the greatest susceptibility, Norway pine from the 24 tests had a total score of 81, white pine 64, jack pine 49, and white spruce 46. This would indicate that Norway pine is in reality most susceptible to heat injury in dry air, with jack pine and white spruce distinctly more resistant, and white pine intermediate.

The influence of light intensity upon killing temperatures at low relative humidities was studied by comparing tests 34 and 35 in darkness with test 36, which had 306 footcandles illumination. The results of these tests were examined carefully to determine whether there were any significant differences due to light intensity. Since other conditions were essentially the same for the three exposures, any difference due to light as such should be apparent. The plants from the three tests were grouped by species and the order of injury determined for each. The number "1" was assigned to the plant receiving least injury, "2" to the next most resistant, and so on. The average scores for the plants in light and those in darkness are given in table 7.

TABLE 7.—*Comparative resistance of young conifers when exposed to the same temperature in light and in darkness*

Species	Mean score	
	Light	Dark
Norway pine	7.0	6.3
White pine	4.5	7.5
Jack pine	7.7	5.9
White spruce	6.7	6.3

Except for white pine there is no significant difference, and even here the difference is little greater than might be expected from chance variation. If light, through its influence on transpiration (*1*), has any important effect upon killing temperature, it was not disclosed by these tests.

DISCUSSION OF RESULTS

For determining the true resistance of conifers to high temperature, the tests in hot water were best, since it may safely be assumed that the parts submerged rather quickly attained the temperature of the water bath. For tests in air, owing both to transpiration, which has been shown to be important even at 88 percent relative humidity (*1*), and to the natural insulation provided by bud scales and bark, the plant parts were probably at a temperature of 2° to 3° C. below that of the surrounding air. Assuming that the killing temperature for the tissue is actually the same in water and air, we may compute the possible temperature difference which prevailed between plant parts and surrounding air. Such values are given in table 8 for 5-hour exposures of Norway pine and white pine. In moist air the killing temperature for buds was at least 3° and for needles 2° above that in water. In dry air the increases in temperature required to kill were more than twice as great except in the case of Norway pine needles.

TABLE 8.—*Increases in external killing temperatures for buds and needles of young Norway and white pines in air as compared with those in water, for 5-hour exposures*

Species	Plant part	Temperature increase	
		In moist air	In dry air
		°C.	°C.
Norway pine	Buds	3.1	6.4
	Needles	2.3	3.6
White pine	Buds	3.5	7.4
	Needles	2.1	7.4

Two factors operate to keep the plant temperature below that of air temperature: (1) Transpiration, and (2) the fact that the plant temperature was below air temperature at the beginning of the test. The greater the specific heat and heat conductivity of the medium in which the plants are exposed, the more quickly will they attain the temperature of the medium, other factors being equal. Consequently, the plants were heated most rapidly in water, and more rapidly in moist air than in dry air. However, in view of the length of the exposures, the rapid circulation of air, and the fact that the plants were also exposed to radiation which would be more effective in dry air than in moist air, it is believed that the more rapid rate of heating, at least in moist air, had little influence on the killing temperature of needles. The dominant factor must have been transpiration. While no especial effort was made to study this specific question in detail, the writer submits the method used as one more likely to give a positive answer to this question than studies in which the high temperatures are produced by excessive radiation alone, or studies of thermal emissivity.

Whether moisture in the air would play any important role in determining the killing temperature for conifers when the soil is dry was not determined. Under such conditions transpiration would be considerably reduced, and any cooling value it might have would be lessened. It would be difficult to determine the influence of humidity on killing temperature of plants in dry soil, since in tests as long as 5 hours in duration desiccation might be a more important factor than heat.

Another important factor in determining the highest temperature a plant will withstand is the duration of the temperature. Referring to tables 4 and 5, it is seen that a 5-hour exposure results in death at considerably lower temperatures than a 2-hour exposure. Such a result might be expected and has been reported by other investigators.

The fact that in the submergence tests the needles and buds were about equally resistant to high temperatures while in the tests in air the buds were more resistant probably means that because of superior insulation and lighter color the buds were not heated to as high temperatures as the needles. The difference in killing temperatures in moist and dry air for buds was less than that for needles. Likewise, in the more massive portions of the plant, the live tissue is protected by light-colored insulating bark, which may have prevented overheating.

94768—36——2

That total radiation intensity had a direct effect on the killing temperature is shown by the fact that for plants with dense needles the upper, more exposed ones suffered much greater injury than the lower, shaded ones. In this respect there seemed to be no difference in effect between radiation provided by electric lamps and by electric heaters. At least, the killing temperature in dry air was not appreciably influenced by an addition of radiation within the visible region of the spectrum.

The relative ability of different species to withstand high temperatures is of particular interest. Norway pine, because of its greater resistance to fire and because both it and jack pine grow in hot, dry, exposed places, is ordinarily assumed to be more resistant to excessive heat than the other species, and white spruce is assumed to be least so. While the evidence from these tests is not complete enough to be unmistakably convincing, it indicates that Norway pine is least resistant during the first few years of life and white spruce fully as resistant as any of the other species that were tested.

From the standpoint of the forester and ecologist, one very significant thing shown by the tests is the comparative uniformity of killing temperatures for all four species. This indicates that when excessively high temperatures occur, all of these species are likely to suffer to about the same degree. Under natural conditions, when the temperature is high enough to kill one species, it will probably kill the other three. In choosing which species to plant on exposed sites where high temperatures are likely to occur, equal consideration may be given to all four species, insofar as this factor alone is concerned. Spruce may be expected to survive as well as jack pine or Norway pine. This statement does not, however, apply to drought resistance.

Still more important from the ecological and silvicultural standpoint than the actual killing temperature is the capacity of a plant to recover from severe injury due to high temperature. In this respect Norway pine is again the poorest and white pine the best. The ability to recover depends upon the capacity of the stem—which for all species is most resistant to heat—to send out epicormic sprouts. White pine appears to excel all others in this respect, followed by white spruce, while jack pine shows little tendency to epicormic sprouting and Norway pine practically none. Epicormic sprouting is valuable also in protecting the plants against injuries caused by browsing or breakage.

CONCLUSIONS

From tests of Norway pine, white pine, jack pine, and white spruce 1 to 4 years old for killing temperatures in a water bath, it may be concluded that: (1) Resistance to excessive heat increases with increasing age and increasing size or mass of plant and tissue; (2) tops are more resistant than roots; (3) with 2-hour exposures the maximum temperature that needles can withstand is 49° C.; (4) temperatures as low as 44.3° do not cause severe damage to tops in exposures up to 5 hours' duration, but may result in death to roots; (5) there is little difference among the species tested in their ability to withstand heat.

For comparable plant material the external killing temperature was higher in air than in water and higher in dry air than in moist air.

The maximum temperature which needles withstood for 5 hours' exposure in moist air (relative humidity, 85 percent) was 50° C., while in dry air (relative humidity, 15 percent) they withstood 54°. Exposures of 5 hours in dry air caused little injury at temperatures of 50°.

Resistance to injury in dry air appeared not to be influenced by radiation intensity in the visible region of the spectrum under the conditions prevailing in these tests.

The cooling effect of transpiration was probably the most important factor involved in the greater resistance of plants to heat in dry air.

Jack pine was least resistant to heat in water and Norway pine least resistant in dry air. In neither case were the differences among species of much significance.

Recovery from excessive heat injury was associated with the capacity to send out epicormic shoots from the uninjured stem. White pine and white spruce excelled in this respect, while jack pine and Norway pine were decidedly inferior. From the ecological standpoint this appears to be the only important difference among the species tested.

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DEVELOPMENT–TEMPERATURE CORRELATION IN THE GREEN BUG, TOXOPTERA GRAMINUM[1]

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INTRODUCTION

The rate of development of animals in relation to temperature has received much attention from biologists. When the time required for development is plotted against temperature, it is found that, in general, the period becomes shorter with higher temperature up to near the maximum of possible development, the curve approximating a hyperbola. When, instead of time, the inverse of time is used, an estimate of rate of development is obtained. This is comparable with rate of reproduction or of any other biological process. When this is plotted, with temperature units on the horizontal axis and rate units on the vertical axis, it will be seen that rate increases with temperature until the temperature is unfavorably high. From the lowest temperature allowing development, the rate line seems to rise with increasing steepness to more favorable temperatures, and then to rise as a straight line through most of the range, almost to the upper limit of development. At higher temperatures the line quickly turns downward.

Much of the work on this subject has been summarized by Ludwig (*8*).[2] Most physiologists have leaned toward a curve of gradually increasing steepness for representing the development-temperature relation. The exponential curve of Van't Hoff (*6*) and the modified curve of Arrhenius (*1*) have been much used. These curves fit the increase in rates of many chemical reactions with rising temperature, but in biology they seem to give better results with such processes as digestion and respiration than with development. Krogh (*7*), however, believed that the development-temperature curve was practically a straight line except at the extreme ends.

Entomologists have favored the expression of time-temperature as a hyperbola (Sanderson (*10*)) or of rate-temperature as a straight line (Sanderson and Peairs (*11*); Peairs (*9*); Chapman (*3, pp. 45–64*)). Bliss (*2*), however, used Arrhenius' curve. Shelford (*12, p. 180*) suggested that a straight line could be used in the "medial" range, but not at the extremes.

EXPERIMENTAL PROCEDURE

This paper presents an analysis of data which were obtained at the Minnesota Agricultural Experiment Station in 1927 in a study of the green bug (*Toxoptera graminum* Rond.). The rearing has been described by the writer (*13, p. 330*), and the temperature cabinets used have been described by Chapman (*3, p. 41*). The thermostatic

[1] Received for publication Mar. 11, 1936; issued October 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 266.

control was not then so well developed as with the equipment described, but was satisfactory and yielded comparable results. Groups of these aphids were reared from birth to maturity at constant temperatures, ranging from 7° to 33° C. A total of 404 wingless individuals were reared. The time required ranged from 5 to 37 days, depending largely on the temperature.

The time for each individual was expressed as a whole number of days; while precision might have been improved by using records in fractions of days, general tendencies are well shown. Rate was expressed in thousandths of total development per day; thus, an aphid requiring 30 days to develop had a rate of development expressed as 33.3, while one requiring 8 days had a rate expressed as 125.0. Either all the individual cases or the means of groups reared at each temperature, when plotted, give the usual hyperbola form for time-temperature. The curve for rate-temperature is also the typical one, with the slightly flattened threshold, the nearly straight line through a large part of the range, and lessening slope and quick downturn toward the upper limit. Data on time and rate of development are given in table 1.

TABLE 1.—*Time and rate of development of Toxoptera graminum, by individual cases, at various constant temperatures*

Time	Rate (1,000÷ days)	Number of individuals reared at—											
		7° C.	10° C.	12° C.	14° C.	17.5° C.	20° C.	22° C.	23° C.	26° C.	30° C.	32° C.	33° C.
37 days	27.0		2										
36 days	27.8	2	1										
25 days	28.6	0	5										
34 days	29.4	0	3										
33 days	30.3	0	5										
32 days	31.2	0	7										
31 days	32.3	1	8										
30 days	33.3	1	4										
29 days	34.5		4										
28 days	35.7		3										
27 days	37.0		1	1									
26 days	38.5		1	1									
25 days	40.0			0									
24 days	41.7			0									
23 days	43.5			1									
22 days	45.5			1	1								
21 days	47.6			2	2								
20 days	50.0			11	5								
19 days	52.6			4	5								
18 days	55.6				19								
17 days	58.8				10								
16 days	62.5				4								
15 days	66.7				2								
14 days	71.4				1	1						1	
13 days	76.9					2						0	
12 days	83.3					21						0	
11 days	90.9					19						0	
10 days	100.0					9	4					0	
9 days	111.1					1	20	1	1			1	
8 days	125.0						22	6	8		4	2	
7 days	142.9						5	14	23	18	5	3	2
6 days	166.7							2	6	12	41	10	
5 days	200.0									1	21		
Total		4	44	21	49	53	51	23	38	31	71	17	2

At the same time there were reared 103 specimens of the winged form, which is found with the wingless in many families of this species. These were not used in the analysis, as they differed slightly from the wingless forms, and their smaller numbers made a separate study

undesirable. Their rate-temperature curve was of the same form as that of their wingless sisters, but of slightly different slope; at most temperatures the time of development differed significantly from the time required by the wingless form. The rate of reproduction (*13, p. 333*) showed a curve of the same general form, but with more marked curvature, a peak at a lower temperature (about 22° C.), and a longer declining phase. These data were not suited in numbers and arrangement for statistical study.

EXPERIMENTAL RESULTS

Above 33° C. nymphs failed to mature, although some lived for several days. A few signs of development were shown a little below 7°, but difficulty of rearing at this temperature made it seem unlikely that any would mature at lower temperatures. At 7° only 4 were successfully reared; many died during development. At 33° only 2 were reared. At 32°, 17 were reared after a number of trials, but many failed to mature and others were retarded considerably in development, generation rearing was difficult, and decreased vigor was shown in several ways. At each of these three temperatures only a minority of those started were reared, while at the intervening temperatures all or nearly all matured. The few maturing at the extreme temperatures may have been a selected group, and the data therefore seem unsafe, both statistically and physiologically, to use in a critical study of the curve of developmental rate and temperature.

The remaining individuals, 381 in all, were reared at nine constant temperatures from 10° to 30° C. The results seemed to be quite normal; of the nymphs started, a large majority matured. While at 30° adults showed depressing effects of high temperatures in shortened life, increase was rapid and generation rearing not difficult. Those reared in this range of temperatures were accordingly studied to determine the best temperature-development curve, the criterion being the standard error of estimate as defined by Ezekiel (*4*). By dropping the few extreme cases the flattening at the threshold and the downturn at the upper end are eliminated, and a nearly straight line is obtained, with slight increase in steepness in the lower part and a slight decrease in the upper part.

STATISTICAL ANALYSIS

The curve of increasing steepness worked out by Arrhenius (*1*) was tried first. The preliminary test of fit for this curve is the plotting of the logarithm of the rate on the horizontal axis and the reciprocal of the absolute temperature on the vertical. If the curve is adapted to the data, this plotting should give a straight line sloping upward from left to right. The means were used in this test, and the plotting indicated that the curve was not adapted to the material. The line showed a definite bend in the middle, but even when divided at the bend the resulting two lines did not promise a good fit. The "Q 10" curve of Van't Hoff seemed even less likely to fit.

The next test was with a logarithmic curve (fig. 1). It is well adapted to gently curving lines, and can be fitted by least squares in the same manner as a straight line, except that the logarithms of one or both variables are used instead of the absolute values. The logarithms may be plotted as a preliminary test, a straight line indi-

cating a probable fit. In this case the logarithms of the mean rates were plotted against the arithmetical values of the temperatures, and vice versa, and a third plotting of the logarithms of both quantities was made. All these test plottings showed the tendency, already noted, to change in slope in the middle; none gave promise of markedly good fit, but the use of logarithms on both sides gave the best

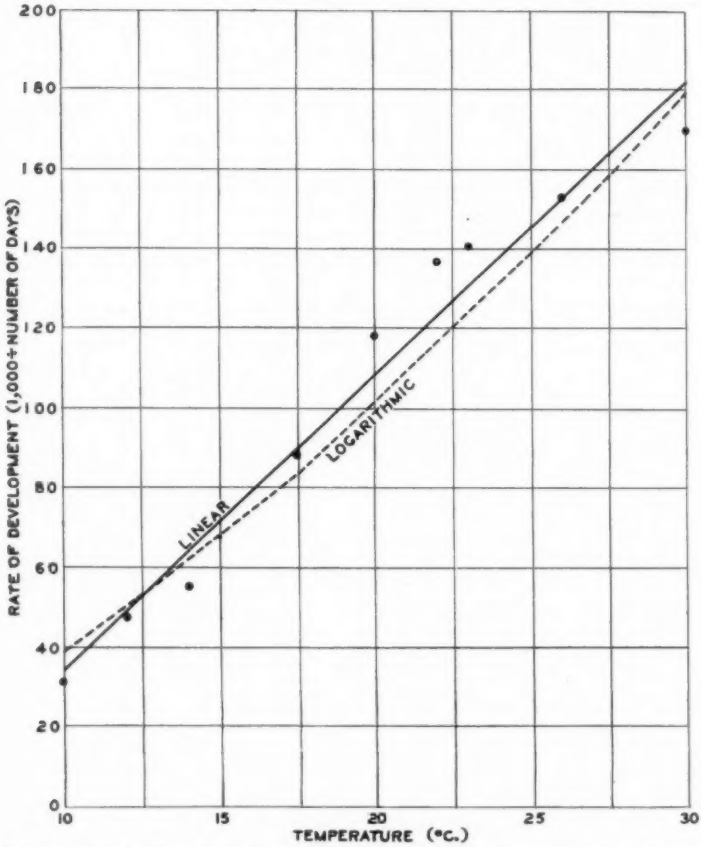

FIGURE 1.—Straight-line and logarithmic curves fitted to rate of development of *Toxoptera graminum*.

promise. The use of logarithms of rates against arithmetical temperature values gave a nearly straight line in the material reared at from 10° to 20° C. Accordingly, the double logarithmic curve was worked out by least squares from the individual cases,[3] and gave a fair fit with a nearly straight curve of very slightly increasing steepness. The formula was $\log Y = 0.200974 + 1.391 \log X$, and the standard error of estimate 16.75 thousandths of total development per day.

[3] For convenience, only means are shown in the various figures.

Next was fitted a freehand curve of a single bend, with slowly increasing steepness, according to the method described by Ezekiel (*3*). In this case the standard error of estimate was 18.94 thousandths of total development per day.

A straight line (fig. 1) was fitted by the method of least squares from individual cases, the formula being $Y=-38.07+7.34X$. A

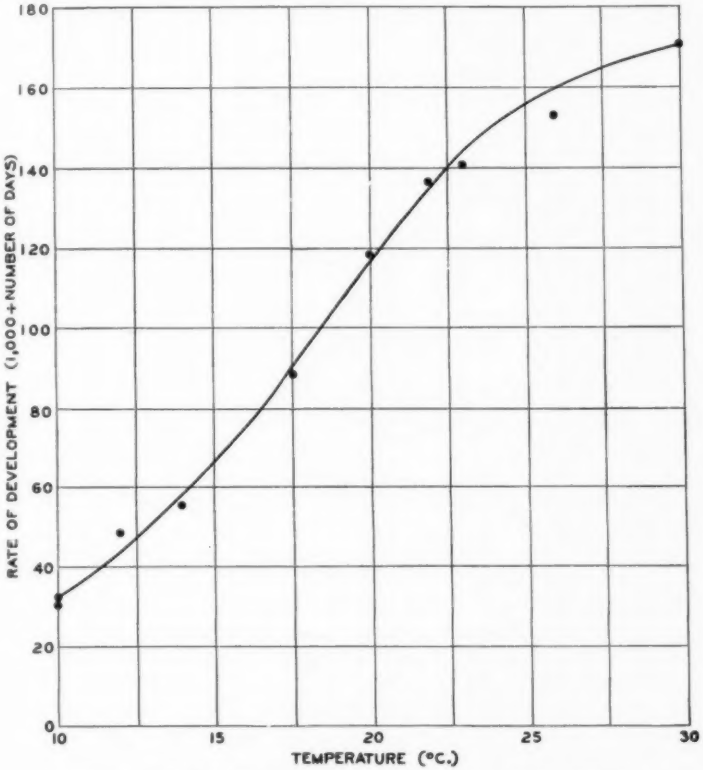

FIGURE 2.—Freehand S-curve fitted to rate of development of *Toxoptera graminum*.

better fit was obtained than with either of the other curves, the standard error of estimate being 14.56 thousandths of development per day.

A freehand curve (fig. 2) rising with increasing steepness to about 20° C., and then inflected to decreasing steepness, or in the form of a very elongated, tilted S, was then tried. This gave a standard error of estimate of 12.85 thousandths of total development per day, the lowest of any curve tried.

Errors of the standard errors of estimate given $\left(\frac{\text{S. E.}}{\sqrt{2n}}\right)$ are in the neighborhood of 0.5 to 0.7, indicating differences significant or nearly so, the significance of the difference between the last two being

most doubtful. The freehand concave curve is significantly the poorest, while the logarithmic curve, which is nearly straight, is a poorer fit than a straight line.

A straight line seems definitely better to represent this relation than single-bend curves of increasing steepness. It appears, however, that some curvature may be present. In view of the advantage the

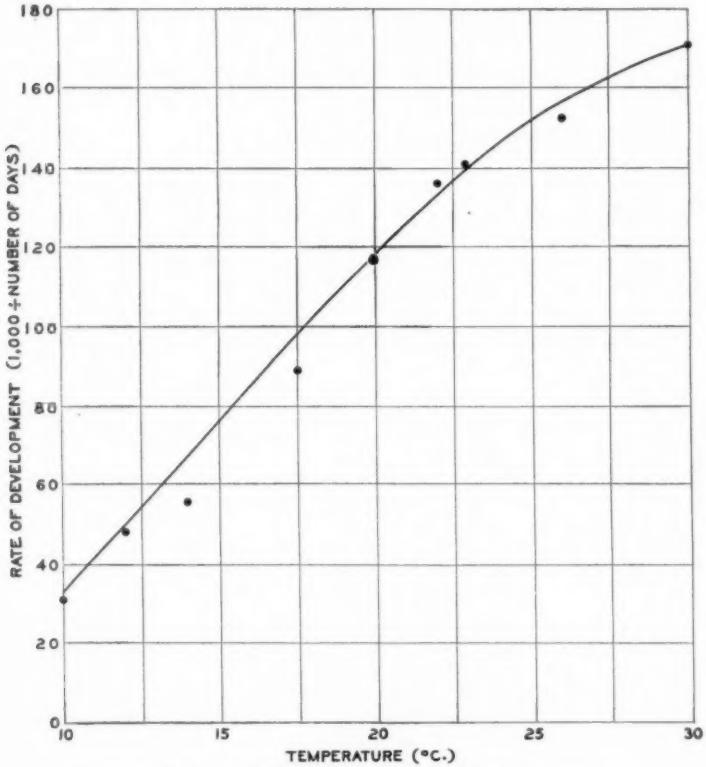

FIGURE 3.—Cubic parabola fitted to rate of development of *Toxoptera graminum*.

S-curve shows over a straight line, this possibility was further tested. When the material was divided and portions were studied separately, it was found that at from 10° to 20° C., inclusive, a concave curve was somewhat better than a straight line; while from 20° to 30° a convex curve was slightly better than a straight line. Blakeman's criterion of linearity was between 2.5 and 3.0. Both these studies of separate portions and Blakeman's test indicate, as did the comparisons of standard errors of estimate, that the advantage of the S-curve over the straight line approaches significance. Departure from linearity was tested by the method of Fisher (*5*, *p. 216*), and proved significant,

with a Z value of 1.25317 against a 1 percent point Z of 0.487. This is the strongest evidence found in favor of the S-curve, indicating that a definite variation not explained by linear regression or random variability exists. The S-curve seems best fitted to represent this condition.

Finally an effort was made to find a mathematical curve adapted to the material, since such a curve can be more easily described or reproduced than a hand-fitted curve. Several curves were found to fit fairly well within the limits of 10° and 30° C., although when extended outside the limits, they departed widely from the curve form usually associated with such material. One of these curves, a cubic parabola, was selected because it followed the general trend of biological material even beyond the limits. The correspondence was not so exact as to suggest a fundamental fitness, but in general this curve was the best. This parabola rose from 10° to 20° as nearly a straight line, with a slight tendency to curve at the lower end. Above 20° it ascended, with some decrease in slope, to a maximum near 30°. The formula was $Y=-32.6+3.74X+0.373X^2-0.0091X^3$, and the standard error of estimate 13.85 thousandths of total development per day. The curve is illustrated in figure 3.

CONCLUSION

It is concluded that for the material used in this study a straight line is definitely better than a curve of increasing steepness for representing the development-temperature relation. Considerable evidence is found that an elongate S-curve may be better than a straight line. It is possible that, with more material and greater precision, the superiority of the S-curve would be better brought out. A freehand curve of this sort has given the best fit, but a segment of a cubic parabola has also given good results.

SUMMARY

A statistical analysis has been made of the rate of development of the green bug (*Toxoptera graminum* Rond.) in relation to temperature. Several curves have been fitted to developmental rate and temperature, and standard errors of estimate, in thousandths of total development per day, have been calculated.

Arrhenius' curve failed to show fitness. With the other curves the results are as follows: Double logarithmic curve, 16.75; freehand concave curve, 18.94; straight line, 14.56; freehand S-curve, 12.85. Futher statistical tests indicate the likelihood of some curvature. After a study of several mathematical curves, a cubic parabola, which gave a standard error of estimate of 13.85, was chosen. The straight line is a better expression of the development-temperature correlation in this material than concave curves, but considerable evidence is found that an S-shaped curve may be still better.

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THE CALCIUM AND INORGANIC PHOSPHORUS CONTENT OF THE BLOOD SERUM OF SWINE[1]

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INTRODUCTION

The object of this study was to ascertain the normal content of calcium and inorganic phosphorus in the blood serum of swine at various ages, and to determine the effect of a high and a low calcium diet on the amount of these elements. Bohstedt and his associates (*3*)[2] noted differences in these constituents in the blood of pigs when the calcium : phosphorus ratio varied greatly in the rations fed. Loeffel et al. (*14*) found wide differences between the maximum and minimum content of these two elements in the blood of the pig, and stated that the blood plasma of animals receiving the direct rays of the sun were higher in these elements than those maintained in the absence of direct sunlight. Hughes and Hart (*10*) reported an average content in the blood serum of swine of 11.77 mg of calcium and 8.53 mg of inorganic phosphorus in 103 samples taken at random in the California Experiment Station herd. Bethke, Edgington, and Kick (*2*) showed the serum calcium and inorganic phosphorus of pigs about 200 days of age to be 11.1 and 6.4 mg, respectively (average of lots 4, 5, 6, 7, 8, 9, and 10 of the third experiment). The average of some of the figures reported by Dunlop (*6*) are serum calcium, 11.32 mg and inorganic phosphorus in whole blood, 6.93 mg (average of 56 determinations of blood taken from hogs weighing from 50 to 200 pounds, in experiment 4, groups 1, 2, 3, and 6). Howland and Kramer (*9*) collected, summarized, and published much of the early work up to and including that of 1921 on the calcium and inorganic phosphorus content of the serum of plasma of domestic animals. In this summary it was shown that three workers had reported, respectively, the calcium content to be 8.7, 9.6, and 9.7 mg and one had reported the inorganic phosphorus to be 2.3 mg, in 100 cc of the blood serum of the pig.

PROCEDURE

Blood for analysis was secured by cutting off the tip of the tail and collecting from 50 to 60 cc in a small Erlenmeyer flask. It was necessary to kill the very small pigs to obtain a sample large enough for analysis. The blood was allowed to clot, the serum poured off, and then centrifuged. In a few cases where the sample was taken before the pig was allowed to suckle, the blood would not clot; in such cases it was impossible to determine the phosphorus. (It was impossible to obtain a clear solution after the addition of trichloracetic acid.) The clear serum obtained after centrifuging was used for analysis. Serum calcium was determined by the Clark-Collip (*4*) modification of the Kramer-Tisdall method and the inorganic phosphorus was ascertained by the method of Fiske and Subbarow (*7*).

[1] Received for publication May 12, 1935; issued October 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 278.

NORMAL SERUM CALCIUM AND INORGANIC PHOSPHORUS OF PIGS AT DIFFERENT AGES

Since the literature indicated that the serum calcium and inorganic phosphorus of the very young animals are greater than those of older and mature animals (an exception is found in the case of chickens as reported by Hughes, Titus, and Smits (*11*), it seemed advisable in attempting to determine the normal blood calcium and inorganic phosphorus of the pig to divide the life span into limited parts or units.

Examples of some of the diets fed, and the calcium and phosphorus content of 100 pounds of the feed mixture, are given in table 1.

TABLE 1.—*Ingredients of the various diets fed and their calcium and phosphorus content*

Composition of diet (pounds)	Calcium	Phosphorus
	Percent	*Percent*
Barley, 43; rice bran, 30; alfalfa, 20; tankage, 5; salt, 1; $CaCO_3$, 1	1.58	0.88
Barley, 88; tankage, 10; salt, 1; $CaCO_3$, 1	1.07	.63
Barley, 52; wheat middlings, 15; tankage, 10; linseed meal, 3; rice bran, 15; alfalfa, 5; salt, 1; $CaCO_3$, 1	1.28	.78
Barley, 58.5; wheat middlings, 25; wheat bran, 14; salt, 1; $CaCO_3$, 1.5	.69	.52
Barley, 95.5; casein, 1.5; salt, 1; $CaCO_3$, 1; cod-liver oil (5–10 cc daily)	.50	.39

All pigs had free access to direct sunlight, some had access to pasture, and some were fed in dry lot.

While the diets were not all optimum for calcium and phosphorus as suggested by Simmonds (*15*), Bethke et al. (*2*), and Dunlop (*6*), the ratio of calcium to phosphorus in each case was in favor of calcium. Except for the last diet listed, the calcium and phosphorus were greater than the 0.65 g of calcium and 0.40 g of phosphorus in 100 g of the food mixture suggested by Simmonds as optimal for the rat. It contained slightly less phosphorus than that suggested by Bethke and his coworkers (*2*), but, the amount of vitamin D was optimum.

The serum calcium of 258 samples and the inorganic phosphorus of 232 samples of blood taken from normal pigs of various ages are shown in tables 2 and 3. These data are arranged to show any differences in the mean values for each age group. One determination of calcium in the blood taken from a pig immediately after the umbilical cord was severed is not shown. The serum calcium in this case was 17.32 mg, the highest for any normal pig recorded in these studies.

The range of the calcium for any one age is less than for the inorganic phosphorus. This is shown in the tables and is substantiated by the standard deviation for the means. The average content of serum calcium in these studies was 11.93 mg, which is slightly higher than the figure reported previously by the writer and Hart (*10*). In that instance only the blood of older pigs was examined, which would account for the difference. The calcium content of the blood of very young pigs (birth to 15 days) is higher than that of older hogs. The mean calcium content for all hogs over 70 days of age (216 determinations) was 11.65 mg, with a standard deviation of 0.69 ± 0.022. The mean value for pigs 210 to 365 days of age is slightly higher than for pigs younger or older. In attempting to find an explanation for this

it was noted that in several instances blood samples had been taken just previous to or soon after farrowing. An average of 11 determinations of the serum calcium and inorganic phosphorus taken from sows at this stage were 12.94 and 7.62 mg, respectively. The calcium content of the blood is apparently higher and the inorganic phosphorus is lower just before and immediately after farrowing.

TABLE 2.—*Calcium content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc								Mean [1] calcium in 100 cc	Standard deviation [1]
	9–10	10–11	11–12	12–13	13–14	14–15	15–16	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
Birth to 15	0	0	0	0	5	9	6	20	14.44	0.62±0.066
15 to 70	0	0	6	12	4	0	0	22	12.40	.49± .050
70 to 120	1	5	30	10	1	0	0	47	11.64	.61± .043
120 to 210	1	7	38	12	0	0	0	58	11.57	.55± .036
210 to 365	1	3	28	15	3	0	0	50	11.85	.72± .045
365 to 730	0	6	19	8	2	0	0	35	11.72	.68± .055
730+	1	5	16	3	1	0	0	26	11.40	.66± .062
Total or average	4	26	137	60	16	9	6	258	11.93	.99± .029

[1] From original, not group data.

TABLE 3.—*Inorganic phosphorus content of the blood serum of normal pigs, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc								Mean [1] inorganic phosphorus in 100 cc	Standard deviation [1]
	5–6	6–7	7–8	8–9	9–10	10–11	11–12	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
Birth to 15	0	0	0	7	2	5	0	14	9.37	0.77±0.098
15 to 70	0	4	2	7	3	3	1	20	8.61	1.31± .140
70 to 120	1	6	6	13	14	4	0	44	8.51	1.18± .085
120 to 210	1	3	7	20	14	3	3	51	8.66	1.20± .075
210 to 365	1	4	17	19	4	2	0	47	8.05	.96± .067
365–730	1	11	6	7	4	1	0	30	7.63	1.15± .100
730+	0	2	14	7	2	1	0	26	7.96	.86± .080
Total or average	4	30	52	80	43	19	4	232	8.34	1.19± .035

[1] From original, not group data.

While the inorganic phosphorus varied considerably for any one age, and while the value for very young pigs (birth to 15 days) is higher than for older hogs, the differences in the means are not so great as for the serum calcium. The mean value for inorganic phosphorus without the very young pigs (birth to 15 days) was 8.27 mg. Whether the higher content of serum calcium and inorganic phosphorus in the very young animal is due to the amount of these elements ingested from the milk of the mother, or whether high concentrations are mobilized in utero for the early growth of the body

tissues is not clear. The serum calcium continues to be relatively high during the entire suckling period.

Although calcium in the blood of the mother just before and soon after parturition was higher than normal, the maternal blood was not so high either in this element or in phosphorus as that of the newborn pig.

After the pig has attained an age of about 70 days (weaning time) there seems to be very little change in its serum calcium throughout life (except for periods immediately before and after farrowing). The inorganic phosphorus, which is higher at birth and for a short time thereafter, gradually decreases until maturity.

EFFECT OF A HIGH AND LOW CALCIUM DIET ON SERUM CALCIUM AND INORGANIC PHOSPHORUS OF SWINE

EFFECT OF HIGH CALCIUM DIET

Bohstedt and his coworkers (*3*) showed that the addition of 2 percent $CaCO_3$ to a diet of white corn, wheat middlings, linseed meal, and salt resulted in a higher content of serum calcium in pig's blood than did the basal diet. Dunlop (*6*) reported increased calcium and decreased phosphorus in the serum of pigs fed rations containing 1.62 percent of calcium, with a calcium-phosphorus ratio of 1.0 to 0.34. Bethke et al. (*2*) found the inorganic phosphorus of swine as low as 4.5 mg in 100 cc of serum when the phosphorus in the ration was from 0.31 to 0.33 percent. Hjort (*8*) stated that calcium salts administered orally to dogs caused a marked increase in the serum calcium of normal animals. Kramer and Howland (*13*) showed that increasing the calcium in the diet of rats increased the serum calcium and depressed the phosphorus.

In the studies relating to the effect of a high calcium diet, two methods of administering the calcium were employed: (1) The pigs were fed diets to which calcium carbonate was added; (2) calcium salts in solution or in suspension were administered directly into the pig's stomach, by means of a funnel and rubber tube. The diet fed in the first series of high calcium tests consisted of barley, 55 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent, to which was added 5 percent of $CaCO_3$. The calcium content of this diet was 2.09 percent and the phosphorus 0.51 percent. The time that elapsed from the beginning of the high-calcium feeding to the taking of the first blood samples ranged from 4 hours to 7 days. A summary of the results is given in tables 4 and 5. A few of the extremely high calcium determinations are not included. These will be discussed briefly later.

The mean calcium content is nearly 3 mg higher and the inorganic phosphorus is about 2.5 mg lower than for normal pigs of the same age. None of the calcium determinations was below 12 mg and none of the inorganic phosphorus determinations was above 8 mg. There is a wide range in the values for serum calcium. Some of the higher ones were from pigs in which calcium salts were administered directly into the stomach.

TABLE 4.—*Calcium content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of calcium in 100 cc									Mean[1] calcium in 100 cc	Standard deviation
	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
70 to 120	4	7	0	1	0	0	0	0	12	13.10	0.75±0.103
210 to 365	11	21	16	12	4	4	3	4	75	14.75	1.83± .101
Total or average	15	28	16	13	4	4	3	4	87	14.53	1.80± .092

[1] From original, not group data.

TABLE 5.—*Inorganic phosphorus content of the blood serum of hogs fed a high calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc							Mean[1] inorganic phosphorus in 100 cc	Standard deviation
	3–4	4–5	5–6	6–7	7–8	8–9	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
70 to 120	0	0	0	5	5	1	11	7.18	0.69±0.099
210 to 365	2	19	22	11	4	1	59	5.50	1.01± .063
Total or average	2	19	22	16	9	2	70	5.77	1.15± .066

[1] From original, not group data.

To determine the effect of suddenly increasing the calcium content of the ration, a group of six pigs 8 months of age were fed for a short time on a ration of barley, wheat middlings, wheat bran, and 1.5 percent of $CaCO_3$, and two pigs of the same age similarly bred were fed a normal diet and kept as controls. On April 30, 1932, the $CaCO_3$ of the experimental animals was increased to 5 percent. The calcium and phosphorus in the ration were, respectively, 2.09 and 0.51 percent. Blood samples were taken and analyzed, as shown in table 6.

A third series of experiments designed to show the effect of administering calcium salts directly into the stomach was carried out. Eight pigs about 3½ months of age were used. Some of them were weaned and some were not. In the morning of July 18, 1932, their average blood calcium and phosphorus were, respectively, 11.97 and 7.3 mg. At 4:30 in the afternoon, 25 g of $CaCO_3$ in 125 cc of water was administered to four of the pigs. The others were kept in the same pen as controls. At 8:30 a. m., July 19, blood samples were taken and examined. The average for the experimental animals was calcium, 31.68 mg, and phosphorus, 6.85 mg. The average for the control animals was calcium, 12.41 mg, and phosphorus, 7.98 mg. The blood serum calcium of the experimental animals ranged from 27.01 to 34.26 mg. None of the pigs was fed on July 19, the day of sampling. On July 20 at 8:30 a. m. another sample of blood was

taken. The average serum calcium of the experimental animals was 10.97 mg and that of the control animals 11.32 mg. During the 24-hour interim the excess serum calcium had disappeared from the blood.

TABLE 6.—*Calcium and inorganic phosphorus in 100 cc of serum of a group of pigs 8 months of age when the calcium in the diet was suddenly increased* [1]

Date of sampling	Samples taken	Calcium	Inorganic phosphorus	Date of sampling	Samples taken	Calcium	Inorganic phosphorus
	Number	*Milligrams*	*Milligrams*		*Number*	*Milligrams*	*Milligrams*
May 2	4	17.28	5.79	May 19	6	14.25	5.61
May 3	6	16.35	5.37	May 24	6	14.32	5.69
May 4	6	16.54	4.39	June 15	6	13.52	6.75
May 11	6	15.35	4.96				

[1] 2 controls fed a normal diet had an average of 12.40 mg calcium and 7.51 mg phosphorus per 100 cc serum for the same period.

The evidence as reported in table 4 shows definitely that the serum calcium of the pig can be increased by giving a large quantity of calcium in the diet. As measured by the calcium content of the serum, pigs show a rapid response either to feeding a high calcium diet, or to having calcium salts administered directly into the stomach. While the feeding of a high calcium diet causes an immediate rise in the serum calcium and while continuous feeding of such a diet results in the calcium remaining above normal, after a time the calcium appears to decrease slowly. Apparently the body attempts to return the serum calcium and inorganic phosphorus to normal values but is unable to accomplish this as long as a marked excess of calcium is furnished. Kramer and Howland (*13*) have postulated that a serious high concentration of calcium and phosphorus in the blood is avoided by excretion of the excess of the kidneys, the intestines, or by the deposition of part of these materials as insoluble calcium phosphate in the bones. A single dose of a calcium salt introduced indirectly into the stomach causes a sudden rise, which is followed by a relatively rapid fall in the serum calcium, extending over a period of not more than 24 hours.

That there is a reciprocal relation of the serum calcium and inorganic phosphorus of the blood of the pig under most conditions seems certain. Under normal conditions the blood calcium and inorganic phosphorus fluctuate round a certain mean; but as the calcium content of the diet is raised the serum calcium of the blood increases and at the same time the inorganic phosphorus decreases.

EFFECT OF LOW CALCIUM DIET

As early as 1920 Kramer and Howland (*12*) showed that the calcium of the blood serum of children affected with tetany was far below that of normal adults, or that of placental blood. Six years later Bohstedt et al. (*3*) reported that pigs fed diets containing less than 0.10 percent of calcium had lower blood serum calcium than those fed from 0.50 to 0.80 percent. In 1930 Loeffel and his coworkers (*14*) found that pigs fed yellow corn, soybean-oil meal, blood meal, dried skim milk, and salt, indoors, had lower blood serum calcium and

inorganic phosphorus than those fed the same ration outdoors. Hughes and Hart (*10*) in 1932 reported a decrease in the serum calcium of two sows fed a ration deficient in calcium. Kramer and Howland (*13*) showed that when the phosphorus in the diet of rats was above normal (except when an adequate amount of calcium was fed to counteract the effect), or when the calcium concentration was reduced to a minimal value, there resulted a decrease in the calcium and an increase in the organic phosphorus in the serum of the rat. Benjamin and Hess (*1*) in tests with young rats on a high calcium diet, and Templin and Steenbock (*16*) in tests with mature rats on a low calcium diet, found that the serum calcium of the animals fed a basal diet was lower than that of animals fed the same diet to which had been added various antirachitic agents such as cod-liver oil, ultraviolet irradiation, and irradiated ergosterol.

The object of the experiments described below was to determine whether rations having an inadequate amount of calcium would effect a change in the calcium and inorganic phosphorus content of the blood serum of pigs of different ages, and whether the physical well-being of the experimental animals would be affected. The rations fed were as follows:

Barley, 99 percent; salt, 1 percent.
Barley, 99 percent; sodium bicarbonate, 1 percent.
Barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent.

The percentage of calcium and phosphorus in the first two diets was, respectively, 0.091 and 0.385, while the third ration contained 0.095 percent of calcium and 0.53 percent of phosphorus; the ratio of calcium to phosphorus being roughly 1:4.0 and 1:5.5.

TABLE 7.—*Calcium content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table*

Age of animals (days)	Samples of serum having indicated milligrams of calcium in 100 cc										Mean[1] calcium in 100 cc	Standard deviation
	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
15 to 70			1	3	0	4	2	9	1	20	10.21	1.63±0.174
70 to 120	1	3	6	2	1	2	6	4		25	8.47	2.17± .207
120 to 210				1	2	10	18	15	1	47	10.47	.87± .061
210 to 365			1	1	3	2	10			17	9.56	1.09± .125
365 to 730			4	4	3	0	4	7		22	9.27	1.92± .196
730+	2	13	12	10	9	5	10	6	1	68	7.99	2.05± .118
Total or average	3	16	24	21	18	23	50	41	3	199	9.13	2.02± .068

[1] From original, not group data.

A summary of the findings in these tests is given in tables 7 and 8. The mean value for serum calcium of 199 determinations of blood serum of pigs of all ages was 9.13 mg; the mean of 182 determinations for inorganic phosphorus was 8.44 mg. The value of calcium in this case was more than 2.50 mg less than the normal serum calcium of pigs of the same ages. The serum calcium of 68 samples of

blood taken from animals 2 years old and older is considerably below the mean. This may be accounted for in part by the fact that several older animals were on a low calcium diet for a long period. Of particular interest is the fact that the inorganic phosphorus of young pigs fed a low calcium diet, some of which were still with their mothers, was much lower than that of older hogs, and was far below the normal for pigs of the same age (p. —). Likewise the inorganic phosphorus for hogs 2 years old or over was higher than the average, while the average of the serum calcium for the same individuals was lower.

TABLE 8.—*Inorganic phosphorus content of the blood serum of hogs fed a low calcium diet, the data being presented in the form of a distribution table*

Age of animal (days)	Samples of serum having indicated milligrams of inorganic phosphorus in 100 cc										Mean [1] inorganic phosphorus in 100 cc	Standard deviation
	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	Total samples		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Milligrams*	
15 to 70	2	3	8	3	1	1	------	------	------	18	6.54	1.21±0.136
70 to 120	1	2	7	5	4	1	1	1	------	22	7.45	1.60± .163
120 to 210	------	------	5	9	9	8	8	1	------	40	8.61	1.36± .103
210 to 365	------	1	1	3	5	5	1	------	------	16	8.51	1.19± .142
365 to 730	------	1	3	3	11	2	1	1	------	[2] 23	8.49	1.79± .178
730+	------	------	4	11	11	22	10	3	1	[3] 63	9.17	1.40± .084
Total or average	3	7	28	34	41	39	21	6	1	182	8.44	1.66± .059

[1] From original, not group data.
[2] 1 determination of 14.15 mg included but not shown in the table.
[3] 1 determination of 13.55 mg included but not shown in the table.

As would be expected, the serum calcium and the inorganic phosphorus varied within wide limits. Many of the blood samples were taken soon after the animals were placed on calcium-deficient rations, and therefore some of the samples, though only a small proportion, were about normal, whereas in certain extreme cases they were below 5 mg. The same wide variations are found in the inorganic phosphorus; however, except for pigs under 7 months old most of the results are above the average for normal pigs of the same age. The statements concerning wider variations than for normally fed pigs is borne out by the standard deviation from the mean.

When the older animals were low in serum calcium for a long time it was very difficult to get them pregnant, and they often failed to conceive. This condition may have been due to a deficiency of vitamin A or to the combination of an insufficient quantity of this vitamin and calcium in the diet. Of those that did farrow many had weak undersized pigs that failed to grow normally, apparently because of a lack of normal milk production by their mothers.

The records of several individuals fed calcium-deficient diets for long periods follow.

Two sows, born in September 1929 and bred in November 1930, were fed a basal diet consisting of barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent. Such a diet contains a relatively small amount of calcium but probably enough

phosphorus for normal growth, and physical well-being. It is low in vitamin A. Both sows farrowed in March 1931, and one farrowed again in October 1931. The other was due to farrow in December of the same year but did not. Neither farrowed thereafter, though fertile boars were put into the pens with them. Serum calcium and inorganic phosphorus were first determined before the sows farrowed in March. Since the records of these two sows are very similar, only one (for 7–F–29) is given in detail (table 9).

TABLE 9.—*Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7–F–29, 6–F–29, and 32–F–30 while on the diets indicated*

SOW 7–F–29 [1]

Diet	Date of sampling	Calcium	Phosphorus
	1931	*Milligrams*	*Milligrams*
Basal, from Nov. 10, 1930, to Dec. 20, 1931	Mar. 7	11.35	9.19
	Mar. 16	10.90	8.76
	Apr. 9	11.00	10.10
	June 3	7.55	11.19
	July 24	6.50	8.88
	July 31	6.80	8.62
	Oct. 12	6.29	10.37
	Oct. 13	6.50	9.47
	Oct. 15	5.05	9.76
	Nov. 9	5.75	9.70
	Nov. 10	4.75	10.22
Basal, plus 1.5 percent of $CaCO_3$ from Dec. 20, 1931, to Jan. 5, 1932	Dec. 19	5.00	9.14
	Dec. 21	6.35	8.13
	Dec. 23	6.85	8.81
	Dec. 28	7.90	8.83
	1932		
Basal, from Jan. 5–14, 1932	Jan. 4	9.18	8.96
	Jan. 7	6.23	10.15
Basal, plus 1.5 percent of $CaCO_3$ from Jan. 14–27, 1932	Jan. 11	5.11	10.94
	Jan. 15	7.91	10.51
Basal, from Jan. 27 to Feb. 8, 1932 (no sunlight)	Jan. 25	9.78	7.97
	Feb. 8	6.94	10.30
Basal, plus 6 drops of viosterol daily from Feb. 8 to Mar. 29, 1932	Feb. 15	7.05	10.52
	Mar. 1	8.22	10.57
	Mar. 8	8.21	----------
	Mar. 25	8.52	----------
Basal, from Mar. 29 to Dec. 22, 1932	Apr. 20	10.61	7.77
	May 15	7.25	8.42
	June 14	6.06	9.20
	July 11	6.55	8.89
	Sept. 13	8.00	9.76
	Dec. 22	8.48	12.55

SOW 6–F–29

	1931		
Basal, from June 14, 1931, to Feb. 18, 1932 (no sunlight)	Oct. 15	10.10	7.08
	Nov. 9	6.15	9.14
	Nov. 10	4.95	9.52
	Dec. 9	5.55	10.00
	Dec. 19	5.90	8.04
	1932		
	Jan. 11	5.56	9.49
	Feb. 8	7.46	9.14
	Feb. 15	5.72	7.87
	Feb. 18	7.25	[2] 11.62

[1] This sow farrowed a normal litter of 8 pigs Mar. 15, 1931, that weighed 22 pounds. The pigs were weaned May 15, of the same year.

[2] The blood sample taken immediately after death.

TABLE 9.—*Calcium and inorganic phosphorus in 100 cc of blood serum of sows 7–F–29, 6–F–29, and 32–F–30 while on the diets indicated*—Continued

SOW 32-F-30 [3]

Diet	Date of sampling	Calcium	Phosphorus
	1931	*Milligrams*	*Milligrams*
Normal herd ration	July 31	11.50	7.06
	Dec. 19	11.45	7.15
	1932		
	Apr. 21	12.02	7.19
	May 9	7.19	8.91
	May 15	6.51	8.29
Basal (barley, 60 percent; wheat middlings, 25 percent; wheat bran, 14 percent; and salt, 1 percent) from Dec. 19, 1931, to Oct. 4, 1932 (no sunlight).	May 23	6.55	8.00
	May 31	7.53	8.29
	June 14	7.43	9.41
	July 11	6.45	8.12
	Oct. 4	5.30	9.47

[3] On Apr. 9, 1932, farrowed 10 normal pigs; late in May became very nervous; by June 14 all the pigs had died.

Not until these sows lactated did their blood calcium show a marked reduction below normal. One of them showed a lack of coordination of the hindquarters when the pigs were weaned and for a time was unable to get up alone, but she gradually regained her strength and after 5 days was able to walk again. The serum calcium of sow 7–F–29 became very low in the fall of 1931. The addition of 1.5 percent of $CaCO_3$ resulted in an immediate but slow rise in the serum calcium. A return to the basal diet caused a decrease in the calcium content of the serum at once. The giving of 6 drops of viosterol daily in the absence of sunlight caused a gradual rise in the serum calcium. A change back to the basal ration, however, resulted in a lowering of the serum calcium during the summer. By late December 1932 both sows were in such bad condition that they were killed. Neither was pregnant.

When the pigs from these sows were weaned in May 1931, they were fed the same basal ration as their mothers, and this diet was continued until August 5. At no time after weaning did the pigs appear normal. Their condition was poor, their hair was dry and curly, and they seemed to have an abnormal desire for water. After August 5, they were divided into two groups. One group was fed the basal diet and the other was fed the basal diet to which was added 1.5 percent of $CaCO_3$. Neither group did well, and by September 13 only one pig in each group was alive. The rest of the pigs had died either of pneumonia or what seemed to be necrotic enteritis. The serum calcium and inorganic phosphorus of these pigs were below normal for animals of their age. From September 13, 1931, to January 7, 1932, the two pigs still alive were fed the basal ration plus 1.5 percent of $CaCO_3$ and each was given 10 cc of cod-liver oil daily. They were finally turned out to pasture, fed a normal diet, and marketed.

The second litter of the other sow of the pair was farrowed October 8, 1931, and the pigs were weak at birth. This sow had little or no milk when the pigs were born; however, on December 12 five of

the seven pigs born were weaned. After weaning they were put on the same basal ration as their mother, and by February 1, 1932, all of them had died. The average serum calcium and inorganic phosphorus of these pigs were: On November 25, 1931, calcium, 7.53 mg and phosphorus, 6.32 mg; on December 12, 1931, calcium, 7.03 mg and phosphorus, 6.40 mg; and on February 10, 1932, calcium, 6.82 mg and phosphorus, 6.25 mg. These pigs, like the others, were far below normal in serum calcium and inorganic phosphorus. The milk of the mother was not tested for calcium or phosphorus, and it is therefore impossible to postulate that the calcium and phosphorus content of the mother's milk was not normal; however, the evidence indicates that such was the case.

The record of a third sow, 6–F–29, shown in table 9, is presented because some of her pigs apparently died of calcium tetany. She farrowed a normal litter in September 1930, and another litter in March 1931. On June 14, 1931, she was put on the same low calcium diet that had been given to sow 7–F–29, and kept away from the direct rays of the sun. On September 18, 1931, she farrowed eight pigs that appeared normal at birth. Like sow 7–F–29, her blood calcium was very low, particularly during the lactation period, and, generally speaking, as her serum calcium decreased her inorganic phosphorus increased. Of the pigs farrowed on September 18 only one was alive by December 21. The rest had died in convulsions. These pigs while apparently still in good condition would act in an abnormal manner, and finally would lie down, thrash about, their muscles would contract, their legs would extend straight out from their body, and death would ensue. Their muscles did not relax after death. The blood calcium of two of these pigs taken after death was, respectively, 4.65 and 7.40 mg in 100 cc of serum. The serum calcium of the latter pig taken 14 days previously was 5.95 mg. On December 8 the one pig that lived after weaning had a serum calcium and inorganic phosphorus content of 6.25 and 11.68 mg respectively. He failed to grow normally and in March 1932 was killed. On post-mortem examination it was found that one of his hip sockets was being resorbed.

The record of sow 32–F–30 is presented in table 9. On October 2, this pregnant sow seemed to be having nervous and muscular difficulties. A note taken from the original record reads: "She trembles all over, gasps for air, grunts loudly, lies down, jumps up suddenly, sits down with front legs stretched in front of her, and appears short of breath." On October 3 her udder developed rapidly, and on the morning of October 4, a blood sample was taken. By afternoon she was unable to rise. The next day she lay on her right side until about 11 a. m., when she began thrashing about and breathing hard. At 1 p. m. she was dead. On post-morten examination, conducted a few minutes after death, six fetuses were removed and blood samples were taken. The fetuses were outwardly normal and weighed altogether 17.5 pounds. The sow had small infection areas in one lung and a slight necrosis of the small intestine. It is unlikely that either caused death; apparently she died of acute calcium tetany. The average serum calcium and inorganic phosphorus for five of the fetuses were, respectively, 19.58 mg and 17.24 mg.

Since the blood of these unborn pigs was taken after the death of the sow, asphyxiation may have influenced the quantity of inorganic phosphorus present. Collip (*5*) found that the inorganic phosphorus of asphyxiated fetuses was higher than that of normal fetuses and that asphyxiation caused a rise of about 1 mg in the blood of the dog.

The serum calcium of the mother taken on October 4 was less than one-third that of the fetuses taken the following day. Her inorganic phosphorus was only about one-half that of the fetuses she was carrying.

The following record shows the normal serum calcium of a sow and her litter during lactation. Sow 14–S–31 farrowed a litter of nine normal pigs April 9, 1932. The average of three samples of blood taken May 9, May 23, and June 14, 1932, was for the sow, serum calcium, 11.35 mg and inorganic phosphorus, 7.03 mg; for the pigs, serum calcium, 12.19 mg and the inorganic phosphorus, 7.86 mg.

SUMMARY

The mean serum calcium and inorganic phosphorus of the blood of normal pigs of all ages was found to be 11.93 and 8.34 mg respectively.

The serum calcium and inorganic phosphorus of the young pig was higher than for the older pig or the adult hog.

The serum calcium of the blood of the sow immediately before and after farrowing was higher, and the inorganic phosphorus slightly lower, than the mean for nonpregnant sows of the same age.

The administration of calcium salts directly into the stomach of the pig caused a temporary rise in the serum calcium.

Continuous feeding of a relatively large proportion of calcium carbonate in the diet caused a prolonged rise in the serum calcium.

As the serum calcium increased above the normal there was at the same time usually a decrease in the inorganic phosphorus.

Continuous feeding of a ration with a low calcium content over a long period of time resulted in a marked lowering of serum calcium.

As the serum calcium decreased below the normal there was at the same time a fairly regular increase in the inorganic phosphorus in the serum of mature animals, but in young pigs there was a decrease.

Evidences of calcium tetany were manifested when the serum calcium became very low.

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NUTRITIVE VALUE OF THE PROTEIN OF CAJANUS INDICUS[1]

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INTRODUCTION

In Hawaii, the pigeonpea (*Cajanus indicus*) is a legume much prized for stock feeding but used only occasionally for human food. The agronomic aspects of this legume in Hawaii have been fully discussed by Krauss (*8*).[2] In some other tropical and semitropical countries, notably in India, the pigeonpea constitutes an important human food.

Since the work here reported was begun, two reports dealing with the biological value of the proteins of pigeonpeas have appeared in the literature. Sundaram, Norris, and Subrahmanyan (*17*) have prepared from the decorticated seeds two globulins, one, which they call cajanin, accounts for 58 percent of the total nitrogen of pigeonpeas, and the other, concajanin, accounts for 8 percent. They also found an albumin which constitutes about 4 percent of the total nitrogen. Their summary states that these three proteins contained requisite amounts of cystine; the quantities of cystine reported in their paper—presumably percentages on the ash- and moisture-free basis—are 2.73 for the albumin, 2.24 for concajanin, and 1.41 for cajanin, all determined by the method of Folin and Looney. Slightly different figures were obtained by the Sullivan method. These authors report no feeding experiments.

Another report published 2 years later by Niyogi, Narayana, and Desai (*12*) gives the results of analysis of the mixed globulins of pigeonpeas and biological values for the pure globulins and the whole seed when fed at a 5-percent protein level by the Mitchell method. In the summary of their article these authors state that the globulins have been found to be deficient in cystine and tryptophane. Their report contains a table giving the quantities of the important amino acids, which it definitely states are expressed as percent of protein—ash- and moisture-free. Cystine is given as 1.58 percent.

Considering the quantities of cajanin and concajanin, which the first-mentioned authors report, one may conclude that the mixed globulins prepared by the two groups contain very nearly the same quantity of cystine, but that the authors of the two reports differ in their interpretation as to what constitutes a "requisite" amount of cystine. However, the quantity of cystine reported in both papers seems very high for legume proteins. The cystine in a number of legume-seed globulins has recently been redetermined by the Bureau of Chemistry and Soils, United States Department of Agriculture, and in no case was it found to exceed 0.58 percent, the average for seven globulins being 0.35 percent.[3]

[1] Received for publication Feb. 25, 1936; issued October 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 292.
[3] D. Breese Jones. Personal communication.

Both reports agree that tryptophane is low in the important globulin, cajanin, and in the mixed globulins; the figure for the latter on the ash- and moisture-free basis being 0.46 percent.

It is usually considered that casein has the requisite amount of cystine, although Sherman and Woods have shown it to be the first "limiting" amino acid (*15*). The cystine content of casein is given variously as 0.20, 0.30, 0.26, and 0.66 percent according to the method used for the determination (*19*).

The work here reported deals with the nutritive value of a meal prepared from the whole (undecorticated) seed of pigeonpeas grown in Hawaii; the preparation and analyses of the mixed globulins prepared from this meal; the results of feeding experiments designed to illustrate the growth and reproduction of rats fed the seed meal as the sole source of protein; and the results of using the paired-feeding method of Mitchell (*11*) in which the diet of one animal is supplemented with one or more amino acids, in this case cystine, and for one small series of experiments, cystine and tryptophane.

A variety of pigeonpea grown on the University Farm and known as strain D was used throughout the tests.

PREPARATION AND ANALYSIS OF GLOBULINS

Preliminary trials showed that under the conditions and temperatures at which it was necessary to work, the greatest quantity of protein could be extracted by using the procedure described below.

Twelve liters of 5-percent sodium chloride solution were added to 2,400 g of finely ground seed meal and stirred continuously with a wooden paddle for one-half hour. The mixture was allowed to stand for 3½ hours, after which it was strained through two thicknesses of cheesecloth and the remaining liquid pressed out in a small strong hand press until the residue was almost dry. No second extraction of the meal was made. The extract, which appeared muddy and opaque, was allowed to stand for an hour or longer and the liquid was decanted from the starch and other materials which settled to the bottom of the flasks.

By putting the decanted liquid through thick pads of paper pulp on large Büchner funnels 10 or more times, an almost water-clear, though somewhat opalescent, filtrate was obtained. Approximately 10½ liters of the clear filtrate were obtained from each lot of 2,400 g of seed meal so extracted.

Considering the conditions for working and the quantities of material involved, dialysis of the filtrate was impractical and dilution and slight acidification were used. To each 1,000 cc of the filtrate, 4 cc of 10-percent acetic acid and 5 liters of distilled water were added, the mixture was stirred thoroughly, placed in large precipitating jars, and kept in a refrigerator overnight.

The following morning the water was siphoned off from the layer of precipitated protein and more distilled water was added. The second morning after siphoning off the water, several lots of protein were usually combined, 95-percent ethyl alcohol was added, and the mixture allowed to stand for 24 hours. The alcohol was removed by decanting and filtering, fresh 95-percent alcohol added, and the mixture again allowed to stand 24 hours. After four such successive washings with 95-percent alcohol, the protein was given one washing

with absolute alcohol which was removed after 24 hours and redistilled ether added. After 24 hours the ether was removed by suction and the protein was dried in a desiccator over sulphuric acid. Any attempt to shorten the process at any point resulted in an end product that was hard and horny. With the process used, the protein appeared as a fine, grayish-white, amorphous powder. The yield for various lots extracted at different times varied from 3.5 to almost 4 percent of the weight of the meal used.

Analyses of a composite sample representing the mixed globulins of the pigeonpea made up from the product of several sets of extractions showed the protein to have 0.73 percent ash on the moisture-free basis. The following tabulation shows the composition—ash- and moisture-free—of the mixed globulins prepared as described above:

	Percent
Carbon	50. 630
Hydrogen	6. 930
Nitrogen	16. 410
Sulphur	. 450
Oxygen	25. 580
Cystine [4]	. 362
Tryptophane [4]	. 367

[4] Analysis furnished by D. Breese Jones, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Sulphur was determined by the official sodium peroxide method (*1*), cystine by the Sullivan method (*16*), and tryptophane by the method of May and Rose (*10*).

These figures show protein prepared as above to contain about the same quantity of carbon, hydrogen, nitrogen, sulphur, and tryptophane as the globulins of pigeonpeas previously reported, but much less cystine.

FEEDING EXPERIMENTS

GROWTH RESPONSE OF RATS FED COOKED AND UNCOOKED CAJANUS SEED MEAL

In 1921 Waterman and Jones (*20*) pointed out that the digestibility of legume proteins was improved by cooking, and in many later experiments (*3*, *4*, *6*, *7*) showed that rats fed cooked legumes fared better than those fed raw legumes.

The finely ground *Cajanus* seed meal for these experiments was cooked without addition of water by placing a 2-inch layer in a pan on a rack in a pressure cooker and heating for 1 hour at 15 pounds pressure. The product, which was slightly damp, was dried at room temperature and reground.

A group of four rats, weaned at 28 days from mothers on a good breeding diet, was fed for 10 weeks diet no. 1 of the following composition: Raw pigeonpea-seed meal, 90 percent; Osborne and Mendel salt mixture, 3 percent; Crisco, 6 percent; and sodium chloride, 1 percent. In addition, the rats were fed 10 drops (0.24 g) of cod-liver oil and 8 drops of tikitiki (equivalent to 1 g of rice bran) daily except Sunday. Four animals of the same litter were fed diet no. 2 of the same composition except that the seed meal was cooked. In each case the *Cajanus* meal supplied approximately 18 percent of protein in the diet. The contrasting growth curves of the two groups are shown in figure 1. The rats fed the cooked pigeonpea-seed meal gained much more in weight and were in much better condition at the end of the experiment than were the rats fed the raw meal.

GROWTH RESPONSE OF RATS FED COOKED CAJANUS MEAL AS THE SOLE SOURCE OF PROTEIN COMPARED WITH GROWTH RESPONSE OF RATS FED BREEDING DIET

Figure 2 illustrates the growth response of additional animals fed the cooked seed meal diet described above. Separate curves for males and females are shown in comparison with growth curves of normal (breeding) animals in the laboratory.

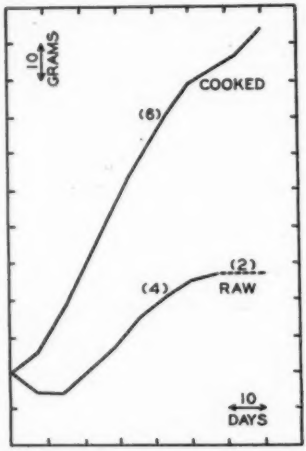

FIGURE 1.—Growth curves of rats fed raw and cooked *Cajanus indicus* meal as the sole source of protein (diets 1 and 2). Figures in parentheses refer to the number of rats used for each test. The broken line indicates the death of one or more rats.

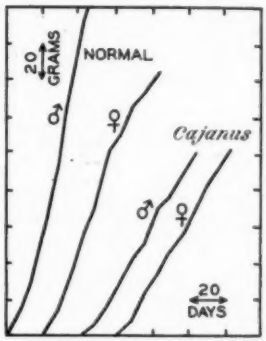

FIGURE 2.—Growth curves of typical rats fed cooked *Cajanus indicus* seed meal (diet 2) as the sole source of protein, compared with growth curves of normal rats fed a good breeding diet containing whole-milk powder.

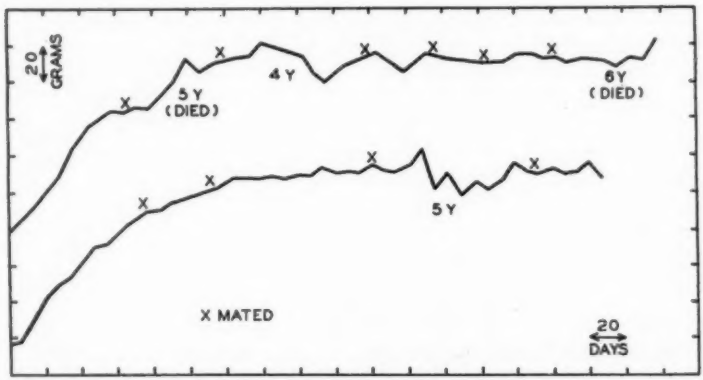

FIGURE 3.—Typical growth curves and breeding record of two female rats fed *Cajanus indicus* meal as the sole source of protein (diet 2). Y means young born.

REPRODUCTION ON CAJANUS MEAL DIET

Four females and two males fed diet no. 2 were used for breeding purposes. Figure 3 illustrates typical growth curves and the breeding record of two of the females fed diet no. 2. Frequent matings resulted in only an occasional pregnancy and the young often died.

GROWTH OF SECOND-GENERATION RATS ON CAJANUS MEAL DIET

Figure 4 illustrates the growth curves of second-generation animals on diet no. 2. They were given five drops of cod-liver oil and 0.2 g of dry yeast daily in addition to the cooked *Cajanus* meal diet. The growth response of these rats is definitely inferior to that of first-generation animals (fig. 2). They did not breed and no third-generation animals could be obtained on this diet.

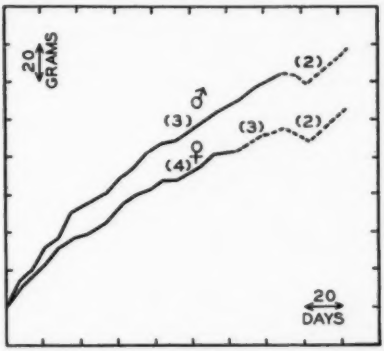

FIGURE 4.—Growth of second-generation rats fed *Cajanus indicus* seed meal as the sole source of protein (diet 2). Figures in parentheses indicate the number of rats used for each test. Broken lines indicate the death of one or more rats.

COMPARISON OF CAJANUS MEAL AND CASEIN AS THE SOLE SOURCES OF PROTEIN

Young rats of the same litters, paired for weight and sex, were placed on the following diets at the age of 4 weeks: Diet no. 3—cooked *Cajanus* meal, 37 percent (furnished 8 percent of protein in the diet); starch, 53 percent; Crisco, 6 percent; and Osborne and Mendel salt mixture, 4 percent. Diet no. 4—casein, 10.2 percent (furnished 8 percent of protein in the diet); cornstarch, 79.8 percent; Crisco, 6 percent; and salt mixture, 4 percent. In addition to the diets allowed ad libitum, 10 drops of cod-liver oil and 0.4 g of dry yeast were fed daily except Sunday. The results of feeding these diets for 9 weeks are summarized in figure 5. The average food consumption of the female rats fed diet no. 3 was about 4 percent greater, and that of the male rats about 10 percent greater, than the average food consumption of the corresponding groups of rats fed diet no. 4.

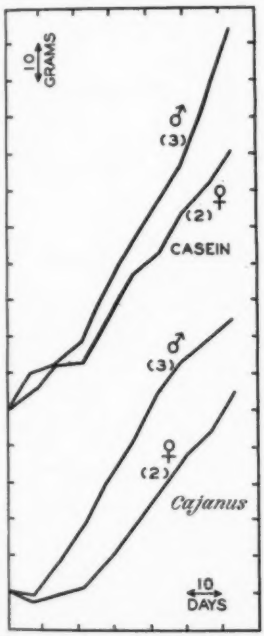

FIGURE 5.—Comparative growth curves of rats fed *Cajanus indicus* seed meal or casein as source of protein (diets 3 and 4 with total protein content equaling 8 percent). Figures in parentheses indicate the number of rats used for each test.

It took the rats fed *Cajanus* meal about a week to adjust themselves to the vegetable-protein diet, but after that they grew at a rate that compared favorably with that of the rats on animal protein.

ARE THE PIGEONPEA-SEED MEAL AND THE GLOBULIN PREPARED FROM IT DEFICIENT IN CYSTINE?

Paired-feeding experiments were made to determine the effect of adding cystine to a diet in which pigeonpea-seed meal furnished the sole protein. Mitchell and his co-workers have shown (*2*, *11*) that most legumes are deficient in this amino acid. The composition of the diets is shown in table 1.

TABLE 1.—*Composition of rations*

Diet and percentage of protein	*Cajanus* seed meal [1]	Crisco	Salt mixture [2]	Cornstarch	Cystine	Sodium chloride	Cod-liver oil	*Cajanus* globulins	Butter
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Diet 5, 8 percent	37	6	4	53					
Diet 6, 8 percent	37	6	4	52.7	0.3				
Diet 7, 8 percent	37	6	4	52		1			
Diet 8, 8 percent	37	6	4	51.7	.3	1			
Diet 9, 11 percent	55	6	4	32		1	2		
Diet 10, 11 percent	55	6	4	31.7	.3	1	2		
Diet 11, 10 percent			4	74.4			2	11.6	8
Diet 12, 10 percent			4	74.1	.3		2	11.6	8

[1] Actual percentage of meal varied as moisture content varied. Each lot of cooked meal was analyzed for protein (N × 6.25) and moisture.
[2] Osborne and Mendel (*13*).

Summarized in table 2 are the results of feeding 35 pairs of rats for 10 weeks. Pairs numbered 1 to 11 received diets 5 and 6, and pairs numbered 12 to 35 received diets 7 and 8. These diets furnished 8 percent of protein from the pigeonpea-seed meal. In addition, the rats were fed 0.2 g of dried yeast daily except Sunday.

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*

8 PERCENT CAJANUS INDICUS PROTEIN

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
No. 1:	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Control	447	59	88	29	
Cystine	434	59	103	44	+15
No. 2:					
Control	354	59	85	26	
Cystine	291	52	58	6	−20
No. 3:					
Control	363	53	65	12	
Cystine	359	52	81	29	+17
No. 4:					
Control	478	70	110	40	
Cystine	456	71	105	34	−6
No. 5:					
Control	464	66	98	32	
Cystine	475	64	118	54	+22
No. 6:					
Control	426	58	94	36	
Cystine	418	58	102	44	+8
No. 7:					
Control	510	63	103	40	
Cystine	453	61	93	32	−8
No. 8:					
Control	459	60	74	14	
Cystine	428	58	91	33	+19
No. 9:					
Control	595	57	93	36	
Cystine	482	55	90	35	−1
No. 10:					
Control	558	52	84	32	
Cystine	488	48	87	39	+7
No. 11:					
Control	377	51	66	15	
Cystine	496	51	82	31	+16
No. 12:					
Control	540	60	96	36	
Cystine	540	63	103	40	+4
No. 13:					
Control	531	56	95	39	
Cystine	531	54	90	36	−3

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

8 PERCENT CAJANUS INDICUS PROTEIN—Continued

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
No. 14:					
Control	534	56	81	25	
Cystine	534	59	91	32	+7
No. 15:					
Control	422	57	72	15	
Cystine	422	56	66	10	−5
No. 16:					
Control	544	56	91	35	
Cystine	544	56	102	46	+11
No. 17:					
Control	506	50	84	34	
Cystine	506	57	78	21	−13
No. 18:					
Control	536	50	83	33	
Cystine	536	51	83	32	−1
No. 19:					
Control	480	56	85	29	
Cystine	477	56	90	34	+5
No. 20:					
Control	458	50	82	32	
Cystine	459	50	94	44	+12
No. 21:					
Control	360	40	58	18	
Cystine	362	44	65	21	+3
No. 22:					
Control	381	49	65	16	
Cystine	379	50	65	15	−1
No. 23:					
Control	376	52	62	10	
Cystine	376	54	70	16	+6
No. 24:					
Control	354	50	60	10	
Cystine	354	49	56	7	−3
No. 25:					
Control	429	50	67	17	
Cystine	430	50	62	12	−5
No. 26:					
Control	401	44	58	14	
Cystine	400	44	68	24	+10
No. 27:					
Control	409	62	78	16	
Cystine	414	62	90	28	+12
No. 28:					
Control	384	59	75	16	
Cystine	379	60	75	15	−1
No. 29:					
Control	472	58	91	33	
Cystine	472	56	90	34	+1
No. 30:					
Control	472	58	80	22	
Cystine	472	60	91	31	+9
No. 31:					
Control	424	51	86	35	
Cystine	417	51	70	19	−16
No. 32:					
Control	461	50	74	24	
Cystine	460	50	74	24	0
No. 33:					
Control	446	58	86	28	
Cystine	450	56	98	42	+14
No. 34:					
Control	446	52	83	31	
Cystine	444	52	83	31	0
No. 35:					
Control	435	50	78	28	
Cystine	436	48	82	34	+6

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

11 PERCENT CAJANUS INDICUS PROTEIN

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1:						
Control	532	17.8	68	129	61	
Cystine	531	17.3	66	134	68	+7
No. 2:						
Control	507	16.7	64	124	60	
Cystine	509	18.0	65	138	73	+13
No. 3:						
Control	471	16.4	62	108	46	
Cystine	474	17.5	62	137	75	+29
No. 4:						
Control	442	16.4	58	106	48	
Cystine	443	16.4	60	107	47	−1
No. 5:						
Control	459	16.3	58	103	45	
Cystine	459	17.0	59	118	59	+14
No. 6:						
Control	509	16.7	57	110	53	
Cystine	509	16.8	57	134	77	+24
No. 7:						
Control	435	15.4	51	98	47	
Cystine	438	16.3	54	110	56	+9
No. 8:						
Control	483	16.7	63	116	53	
Cystine	485	17.6	61	135	74	+21
No. 9:						
Control	450	15.8	55	108	53	
Cystine	450	16.0	55	112	57	+4
No. 10:						
Control	488	16.8	60	111	51	
Cystine	489	17.1	62	121	59	+8
No. 11:						
Control	462	16.5	53	99	46	
Cystine	462	15.4	48	97	49	+3
No. 12:						
Control	500	16.3	57	110	53	
Cystine	500	15.8	64	103	39	−14
No. 13:						
Control	474	15.5	54	94	40	
Cystine	474	15.9	50	104	54	+14
No. 14:						
Control	436	15.8	49	99	50	
Cystine	440	16.2	47	103	56	+6
No. 15:						
Control	458	16.4	51	108	57	
Cystine	448	16.1	48	102	54	−3
No. 16:						
Control	429	15.5	44	94	50	
Cystine	429	15.8	47	100	53	+3
No. 17:						
Control	467	14.8	42	94	52	
Cystine	471	16.3	42	100	58	+6
No. 18:						
Control	365	16.4	69	105	36	
Cystine	365	17.1	69	115	46	+10
No. 19:						
Control	353	15.9	67	103	36	
Cystine	353	17.1	66	107	41	+5
No. 20:						
Control	325	15.8	58	95	37	
Cystine	325	15.3	56	96	40	+3
No. 21:						
Control	337	15.8	62	86	24	
Cystine	337	16.2	61	107	46	+22
No. 22:						
Control	343	16.0	61	96	35	
Cystine	343	15.8	61	103	42	+7
No. 23:						
Control	388	16.8	66	110	44	
Cystine	388	16.7	66	129	63	+19
No. 24:						
Control	392	16.2	59	131	72	
Cystine	392	16.7	61	127	66	−6
No. 25:						
Control	316	15.4	59	86	27	
Cystine	316	15.6	58	97	39	+12

TABLE 2.—*Supplementing effect of cystine on rations containing 8 and 11 percent of Cajanus indicus protein in the form of a seed meal*—Continued

11 PERCENT CAJANUS IDICUS PROTEIN—Continued

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
No. 26:						
Control	319	15.6	56	87	31	
Cystine	319	15.1	55	96	41	+10
No. 27:						
Control	388	17.6	65	137	72	
Cystine	388	17.2	65	133	68	−4
No. 28:						
Control	422	17.1	60	131	71	
Cystine	422	16.7	59	126	67	−4
No. 29:						
Control	461	17.5	54	138	84	
Cystine	461	16.6	54	120	66	−18
No. 30:						
Control	380	16.4	52	111	59	
Cystine	380	16.2	52	114	62	+3
No. 31:						
Control	379	16.5	63	125	62	
Cystine	379	16.5	62	122	60	−2
No. 32:						
Control	469	17.1	60	124	64	
Cystine	469	18.2	61	151	90	+26
No. 33:						
Control	406	16.5	55	106	51	
Cystine	406	16.7	56	125	69	+18
No. 34:						
Control	380	16.2	52	109	57	
Cystine	380	15.7	52	107	55	−2

The results of these experiments would indicate that cystine has only a slight supplementary value. A statistical analysis of the results by Love's modification of Student's method (*9*) shows that the odds are somewhat greater than 25:1 in favor of cystine. The food consumption of the first 11 pairs is not so uniform as it should be, the total intake varying considerably in several cases. If these 11 pairs are eliminated and the statistical analysis made on the remaining 24 pairs, the odds are only 11:1 in favor of cystine.

It was thought that possibly tryptophane or some other amino acid was the limiting factor when the seed meal was fed at a level to provide 8 percent of protein. Additional experiments were therefore planned in which the seed meal furnished protein equal to 11 percent of the diet. The results of feeding diets 9 and 10 to 34 pairs of rats are summarized in table 2. Pairs numbered 1 to 17 were fed for 8 weeks and pairs 17 to 34 for 6 weeks. Statistical analyses of these results show cystine to have a definite supplementary value, as the odds are greater than 1,110:1 in favor of cystine.

When the prepared pigeonpea-seed meal protein representing the mixed globulins was fed for 8 weeks to 7 pairs of rats at a level of 10 percent (diets 11 and 12), the results were not significant for cystine, as the odds were only 2:1. The results are summarized in table 3. With the same diets (11 and 12), a second set of paired feeding experiments was carried out. At the end of a 5-week feeding period the results were not conclusive, as the odds were only 4.5:1 in favor of cystine. However, when 0.2 percent of tryptophane was added to the diet containing cystine (diet 12) and the same rats were fed for 2 weeks longer the results were very different. A statistical analysis of the results by Love's method showed the odds to be 216:1

in favor of the diet containing the two amino acids. The results, summarized in table 3, indicate very definitely that tryptophane is a limiting growth factor when the pure globulins are fed, but they do not indicate whether cystine is a limiting growth factor. Possibly the rats would have grown as well with tryptophane but without cystine as with both amino acids. Time and materials were not available for additional tests.

TABLE 3.—*Supplementing effect of cystine and of cystine and tryptophane on rations containing 10 percent of Cajanus indicus protein in the form of the mixed globulins*

CYSTINE

Pair	Food consumed	Final body length	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*	*Grams*
No. 1:						
Control	317	15.5	54	82	28	
Cystine	317	15.8	46	86	40	+12
No. 2:						
Control	290	14.5	46	72	26	
Cystine	284	14.7	46	77	31	+5
No. 3:						
Control	317	14.8	44	78	34	
Cystine	317	14.6	44	76	32	−2
No. 4:						
Control	313	15.3	56	84	28	
Cystine	319	15.3	56	80	24	−4
No. 5:						
Control	322	15.8	55	85	30	
Cystine	321	15.6	48	82	34	+4
No. 6:						
Control	309	15.2	53	80	27	
Cystine	307	14.8	53	78	25	−2
No. 7:						
Control	318	14.4	44	68	24	
Cystine	320	14.6	54	72	18	−6

CYSTINE, 5 WEEKS

Pair	Food consumed	Initial weight	Final weight	Total gain	Difference in gain between control and cystine
	Grams	*Grams*	*Grams*	*Grams*	*Grams*
No. 8:					
Control	215	55	72	17	
Cystine	215	55	69	14	−3
No. 9:					
Control	209	54	70	16	
Cystine	209	54	74	20	+4
No. 10:					
Control	193	55	70	15	
Cystine	193	55	70	15	0
No. 11:					
Control	199	53	68	15	
Cystine	199	53	75	22	+7
No. 12:					
Control	190	50	64	14	
Cystine	190	50	66	16	+2
No. 13:					
Control	158	48	54	6	
Cystine	158	48	53	5	−1

TABLE 3.—*Supplementing effect of cystine and of cystine and tryptophane on rations containing 10 percent of Cajanus indicus protein in the form of the mixed globulins*—Continued

CYSTINE AND TRYPTOPHANE, 2 WEEKS LONGER

Pair	Food consumed	Final body length	Final weight [1]	Total gain	Difference in gain between control and cystine plus tryptophane
	Grams	*Centimeters*	*Grams*	*Grams*	*Grams*
No. 8:					
Control	87	15.3	78	6	
Cystine and tryptophane	87	15.7	84	15	+9
No. 9:					
Control	85	15.0	76	6	
Cystine and tryptophane	85	15.2	82	8	+2
No. 10:					
Control	85	15.2	79	9	
Cystine and tryptophane	85	16.1	88	18	+9
No. 11:					
Control	60	15.0	72	4	
Cystine and tryptophane	60	15.6	86	11	+7
No. 12:					
Control	85	14.4	70	6	
Cystine and tryptophane	85	15.3	87	21	+15
No. 13:					
Control	72	13.8	63	9	
Cystine and tryptophane	72	13.8	66	13	+4

[1] Initial weight is final weight in the table above.

DISCUSSION

The tabulation on page 283 shows that the sulphur content of the mixed globulins is 0.45 percent and the cystine content 0.362 percent. The cystine accounts for only 19 percent of the sulphur. In what form does the remaining sulphur exist? If it is in the form of methionine, it is probable that it can be utilized as effectively as though it were in the form of cystine, as Jackson and Block (*5*) have shown that methionine greatly stimulated the growth of rats subsisting on a cystine-poor ration.

An arresting comment on the growth response of rats fed definite amounts of cystine has recently been made by Swift, Kahlenberg, Voris, and Forbes (*18*). They state that when their rats received a total of 0.022 g of cystine per week in their rations, the addition of more cystine did not result in increased growth.

Careful checking of the feeding experiments reported in this paper indicates that all the rats fed the diets without cystine supplements received a rather high intake of sulphur and that, even though not all of it is in the form of cystine or methionine, the intake of these two substances must have been much in excess of 0.022 g per week. For example, in the experiments in which seed meal was fed to provide 11 percent of protein, only about 8 percent of the sulphur would need to be in the form of cystine in order to furnish 0.022 g per week. As the writers have in every case fed 0.2 g of dried yeast daily except Sunday, 0.00324 g of cystine per week would have been added from this source, using the value of 0.270 percent cystine for dry yeast which the above-mentioned authors (*18*) report.

In the experiments reported by Mitchell and Smuts (*11*), if only one-fourth of the sulphur in soybeans were in the form of cystine, the rats would have received a little more than 0.024 g of cystine per week, yet they concluded from their feeding experiments that soybeans are deficient in cystine. The soybean meal used by them was

ether-extracted but not cooked and the rats ate small quantities of the ration. One wonders what the results might have been with cooked soybeans and better food intake. Piper and Morse (*14*) report soybeans to contain 0.444 percent of sulphur. The station pigeonpea-seed meal contained 0.22 percent of sulphur.

SUMMARY

Although first-generation rats made good growth, second-generation rats made only fair growth when pigeonpea-seed meal was fed at a level calculated to provide 18 percent of protein in the diet. Reproduction was obtained with first-generation rats fed cooked *Cajanus* meal, although the record was unsatisfactory. No reproduction was obtained on second-generation rats fed the same diet. Pigeonpea protein is therefore not adequate for normal growth and reproduction of rats.

When pigeonpea-seed meal was fed at a level calculated to furnish 8 percent of protein, the addition of cystine did not materially improve the growth of rats, but when the seed meal furnished 11 percent of protein the addition of cystine markedly improved the growth of rats.

The first limiting growth factor of the prepared pigeonpea globulins appears to be tryptophane.

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STUDIES ON THE INHERITANCE OF RESISTANCE TO WILT (FUSARIUM NIVEUM) IN WATERMELON [1]

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INTRODUCTION

Wilt in watermelons (*Citrullus vulgaris* Schrad.) caused by *Fusarium niveum* (E. F. Smith), takes a heavy toll each year from melon growers in many sections of the United States. Since the causal organism lives over in the soil for as long as 15 to 20 years, the most promising method of controlling the disease is breeding for resistance. However, the existence of many physiological forms of the causal organism, differing in their action on the host (*8*),[3] may necessitate the development of a resistant melon for each locality in which different forms are found. The history of the Conqueror watermelon (*5*) demonstrates that a variety may be resistant in one locality and susceptible in another.

The object of the investigations reported in this paper was to determine if possible how resistance to *Fusarium niveum* is inherited. Similar studies have been made of resistance to other plant diseases, but up to the present very little has been reported on watermelon wilt.

REVIEW OF LITERATURE

Orton (*5*) crossed the citron, which is resistant to *Fusarium niveum*, with the susceptible Eden variety of watermelon and obtained in succeeding generations a resistant melon of good quality, which was called Conqueror. This melon proved resistant in eastern Iowa but was susceptible in Oregon.

Porter (*6*) used citron, African forage, and Chinese melons in crosses with susceptible watermelons. The Chinese melon was resistant, the citron showed 18 percent of wilted plants, and the African forage melon was immune. The F_1 from these crosses was as susceptible as the susceptible parent. Resistance in the F_2 varied, depending on the degree of resistance of the resistant parent. Flesh quality of the resistant parent was dominant in F_1. Porter and Melhus (*7*) in 1929 stated that "the F_3 generation of Kleckley-citron (variety Kafir) crosses proved 86 percent resistant." The F_3 of a Kleckley Sweet × Conqueror cross showed considerable resistance.

EXPERIMENTAL MATERIAL

The parental material consisted of commercial Early Fordhook obtained from a seed firm, and a watermelon from Russia obtained by E. C. Sherwood through the Office of Plant Introduction of the

[1] Received for publication Jan. 7, 1936; issued October 1936. Scientific Paper no. 157 of the West Virginia Agricultural Experiment Station.

[2] The writer wishes to express his appreciation to the following persons: Dr. R. J. Garber, under whose general direction the investigation was carried on, for his counsel and guidance; Dr. C. R. Burnham for suggestions regarding the cytological phase of the study and for help in the preparation of the manuscript; Dr. M. M. Hoover for valuable suggestions contributed during the progress of the investigation; Drs. C. R. Orton, L. H. Leonian, and Bailey Sleeth for aid in the pathological aspects of the problem; F. D. Cornell, Jr., for lettering the charts; and W. E. Rumsey for assistance with the photographic work.

[3] Reference is made by number (italic) to Literature Cited, p. 305.

United States Department of Agriculture. This Russian melon was the one used by Sleeth (*8*) in his studies. It is inedible and has a white flesh which is very tough and bitter to the taste. As compared with the Early Forkhook, the Russian vines grow more vigorously and have longer internodes and coarser leaves. The flowers appear considerably later and the fruits are much longer than those of the Early Fordhook, which are nearly round. The seeds of the Russian are green, while those of Early Fordhook are nearly white. The Russian is resistant to wilt, while the Early Fordhook is susceptible. The Sweetheart variety, obtained from a seedsman, has been used as a check throughout the tests because of its extreme susceptibility.

Three physiologic forms of *Fusarium niveum* (nos. 5, 8, and 20) obtained from Sleeth (*8*), in pure culture were used in the greenhouse tests for the genetic analysis. Sleeth had found that form 5 from

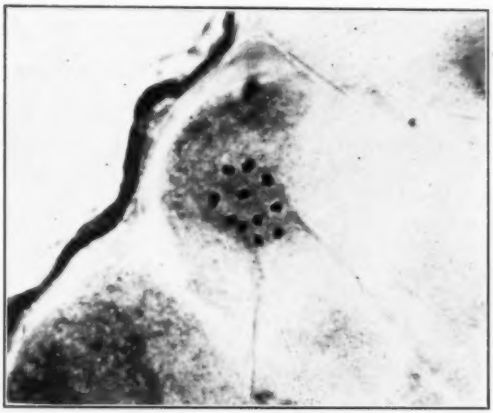

FIGURE 1.—Photomicrograph of diakinesis in a microsporocyte of the Russian watermelon showing 11 pairs of chromosomes. × 970.

Oregon was least virulent, form 20 from North Carolina most virulent, and form 8 from Iowa was intermediate. These particular forms were chosen because they differed in virulence, were distinguishable morphologically, and had proved rather stable in Sleeth's work.

Kozhukhow (*3*) reported 11 pairs of chromosomes for several varieties of commercial watermelons. In the present study an examination was made of microsporoctye material from the Russian and Early Fordhook parents by the iron-acetocarmine smear method (*1*). The chromosome number in both cases was found to be 11 pairs. This correspondence in chromosome number was not unexpected since there was no sterility in the hybrids between the Russian and the commercial variety. At the pachytene stages th chromosomes did not stain very well by the method used, but the best preparations showed the chromosomes to be long, very thin, and considerably twisted. One of them appears to be associated with the nucleolus. At diakinesis the chromosomes are short, as may be seen in figure 1.

METHODS

In 1930 crosses were made at the West Virginia station between the Russian and Early Fordhook for the purpose of developing a commercial watermelon for West Virginia resistant to wilt. In 1932 the writer, with the material at hand, began a study of the mode of inheritance of resistance.

The F_1 was grown in 1931 when selfings and backcrosses to the Russian and Early Fordhook were made. In 1932 the F_2 and certain of the backcross progeny were grown in the field in soil that was artificially inoculated by covering the seeds with a mixture of the soil which Sleeth (*8*) had used in his greenhouse wilt tests. Several forms of the organism coming from various parts of the United States were present. The watermelon plants grown in this soil showed very little wilting. In addition, another planting was made of the Early Fordhook and Russian backcrosses in uninoculated soil. Numerous selfings in both plots were made to produce F_3 and backcross families. There was a possibility that some susceptible genotypes were eliminated in the infested field. This source of error is felt to be of minor importance, however, since very little wilt appeared. Inasmuch as the parent varieties differed in several respects, detailed notes were taken at harvest on shape, flesh color and texture, and seed-coat color.

The genetic analysis of wilt resistance is based on the reaction (in infested soil in the greenhouse) of F_3 families, i. e., the progeny of selfed F_2 plants, and of backcross families, i. e., the progeny of selfed backcross plants. The testing of this material was started in the winter of 1932–33 and continued until the following winter.

Plantings were made in boxes 10 feet by 3 feet by 7 inches, filled with a rich loam soil which had been sterilized for about 4 hours under a large steam plan. After the second planting, sterilized sand was added to the soil to improve its texture.

Inoculum was prepared by growing the forms of *Fusarium* on rice for several weeks. It was then mixed with a bucket of sterile sand and thoroughly incorporated with the surface 3 inches of soil in the boxes. The plan was to run duplicate tests of each family with each of the three forms. Form 5 occupied boxes 1 and 2; form 8, boxes 3 and 4; and form 20, boxes 5 and 6. The boxes were separated from each other by at least 2 feet, and extreme care was exercised to prevent transfer of the infested soil. After working in soil inoculated with a certain form, the writer sterilized his hands for 2 or 3 minutes in 1:1,000 mercuric chloride solution and rinsed them in tap water before working in soil inoculated with a different form.

Before planting, the seeds were sterilized for one-half hour in a 1:1,000 solution of mercuric chloride. Twenty-five seeds were planted about 1½ inches apart in rows 4 inches apart. Each family was represented in each of the six boxes, thus comprising duplicate tests with each of the three forms.

The seed coat of the Russian seeds is much harder and thicker than that of the Early Fordhook, which makes the Russian slower in germinating. Segregation of this character would be expected and would cause some variation in the time of germination among the families.

As soon as wilt began to appear, seedlings showing the wilt symptoms were pulled at 2-day intervals and the number recorded. Throughout the experiment care was exercised to pull the seedlings at the same stage of wilting. Frequent isolations were made from the wilted plants to see whether they contained the form used in the inoculation. The seedlings were sterilized in a 1:1,000 solution of $HgCl_2$ for 5 minutes and then plated on Leonian's (*4*) fusarium agar. In all cases the original form was recovered from the wilted plants.

Thermographic records showed that the air temperature near the boxes fluctuated between 15° and 27° C. in winter and between 15° and 32° in summer. There was an occasional drop to 10° in winter and an occasional rise to 38° in summer. Sleeth (*8*) found that the optimum temperature for the growth of *Fusarium niveum* in culture was between 24° and 28°.

EXPERIMENTAL DATA

In the greenhouse tests of the parents and F_1, the Russian wilted 5.3, 26.1, and 16.7 percent, respectively, to forms 5, 8, and 20. The corresponding percentages for Early Fordhook were 4.5, 95.7, an 95.8; and for the F_1, 14.7, 83,3, and 68.5.

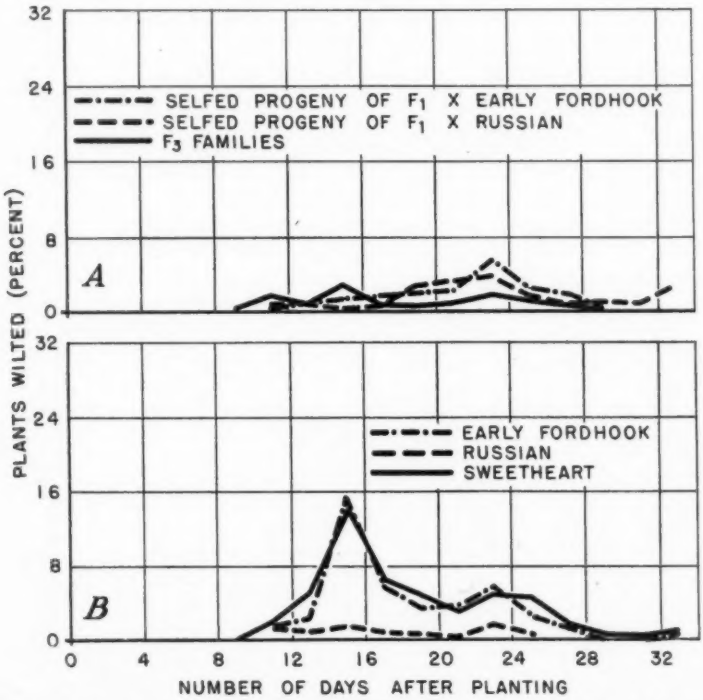

FIGURE 2.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 5 of *Fusarium niveum*: *A*, Families descended from the Russian-Early Fordhook cross; *B*, parents and check.

INCIDENCE OF WILTED PLANTS AMONG PARENTS AND PROGENY

Since all the families could not be tested at the same time, in order to study the rate of wilting it was necessary to have some basis for comparing the results of different tests. Two methods were used. (1) Comparisons were made of the amount of wilt occurring on any given number of days after the first wilt had appeared in those particular tests. The Sweetheart check was used to determine this date of first wilting since it was the most susceptible variety. (2) Comparisons were made of the amount of wilt on any given number of

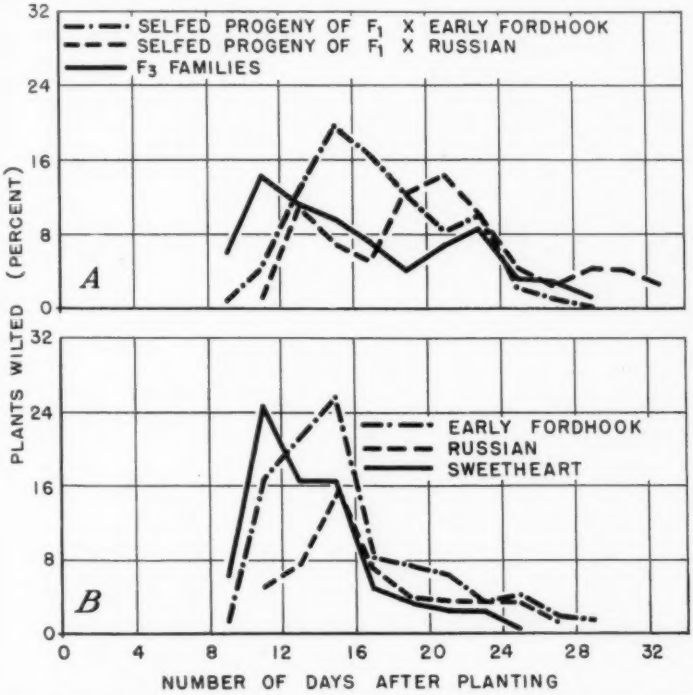

FIGURE 3.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 8 of *Fusarium niveum*: *A*, Families descended from the Russian-Early Fordhook cross; *B*, parents and check.

days after planting. From one test to another there was frequently a variation of several days in the time from planting to the date of first wilting, and the first method was used to eliminate some of the effects of this variation.

The curves showing the incidence of wilt (based on time elapsed after planting) among the parents and Sweetheart (figs. 2, *B;* 3, *B;* and 4, *B*) indicate that in Early Fordhook the wilt proceeded more rapidly than in the Russian. The wilt in the Sweetheart check proceeded even more rapidly than that in Early Fordhook. In all cases there was more wilt in the Early Fordhook and Sweetheart

than in the Russian. This is noticeable particularly in the test with form 5, where there was very little wilt in the Russian (fig. 2, *B*)

Since the wilt (due to forms 8 and 20) that took place in the Russian parent occurred later than that in Early Fordhook, one would expect the families from backcrosses [4] to the Russian to show a delay in time of wilting. The curves (figs. 2, *A*; 3, *A*; and 4, *A*) for the backcross and F_3 families show that this usually is the case. In the families from backcrosses to Early Fordhook the wilt occurred earlier than those in from backcrosses to the Russian. There is a suggestion

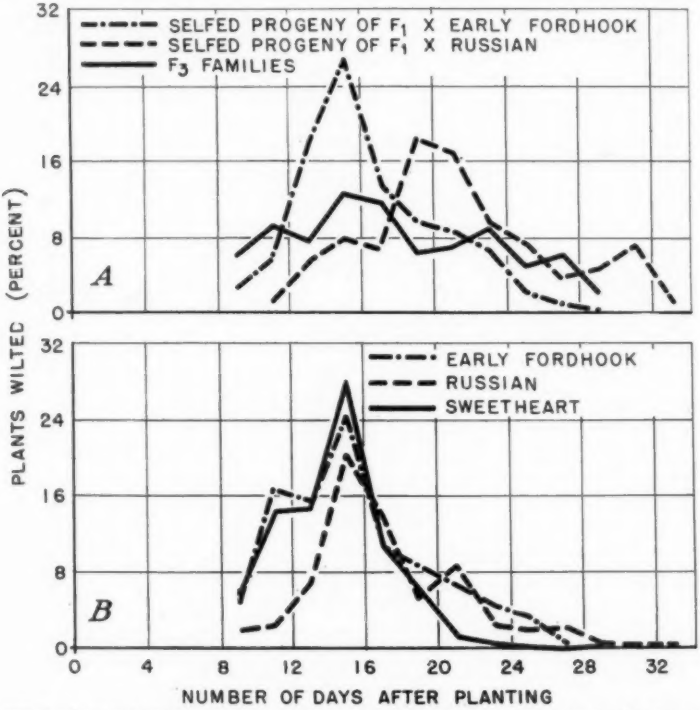

FIGURE 4.—Time and extent of wilt of watermelons when subjected in the greenhouse to form 20 of *Fusarium niveum*: *A*, Families descended from the Russian-Early Fordhook cross; *B*, parents and check.

of bimodalism in the reaction to form 8 (fig. 3, *A*) of the F_3 and the backcrosses to the Russian. This may indicate genetically distinct groups for resistance or a change in susceptibility at different stages of growth. As in the F_1 tests, form 5 caused very little wilt.

Four days after wilting began it was found that the peak of wilting in Early Fordhook had passed, whereas in the Russian it has not yet come. Consequently it was decided that the data on wilting obtained at the end of this 4-day period, in addition to that obtained at the end of the test, would be good material for use in analyzing the genetic difference between the Russian and Early Fordhook parents with regard to resistance.

[4] Throughout this paper the term "backcross" is used to indicate the selfed progeny of the F_1 X parent unless otherwise stated.

DISTRIBUTION OF FAMILIES WITH RESPECT TO PERCENTAGE OF WILTED PLANTS PER FAMILY

One hundred and eighteen families from the $F_1 \times$ Early Fordhook, 62 families from the $F_1 \times$ Russian, and 102 F_3 families were tested for resistance to each of the 3 fusarium forms. The results of these tests are shown in figures 5 and 6.

The tests of the F_3 and backcrosses to each parent were run at different times, which made it necessary to show the reaction of the parents during each test for purposes of comparison. The percentage of wilt is indicated by squares for the Russian parent, by circles for the Early Fordhook parent, and by triangles for the Sweetheart check. The position of the symbols directly above the bars indicates the average reactions of the parents and Sweetheart during the tests of the families which these bars represent. For example, the location of the square above the black bar in the 20–39.9 percent class (fig. 5, *A*) shows that the average percentage of wilted plants in the Russian parent during the tests of these Early Fordhook backcross families to form 20 fell in this class. Likewise it may be seen from this same distribution that the average percentages of wilted plants among the Early Fordhook parent and the Sweetheart variety fell, respectively, in the 40–59.9- and the 60–79.9-percent classes.

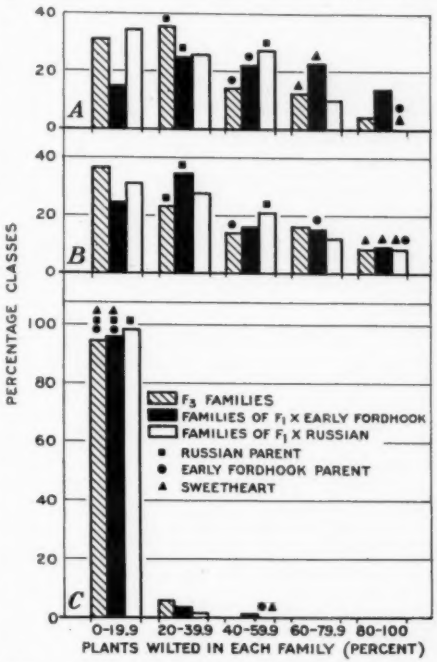

FIGURE 5.—Histograms showing the distribution of families with respect to the percentage of wilted plant per family during the first 4 days after wilting appeared in the tests with the three forms of *Fusarium niveum: A*, form 20; *B*, form 8; *C*, form 5.

The distribution of the F_3 and backcross families with regard to the amount of wilt occurring during the first 4 days of wilting is shown in figure 5. It will be seen that with form 5 practically all the families in the F_3 and backcrosses to the Russian and Early Fordhook are in the lowest wilt class (0–19.9 percent), with some in the 20–39.9-percent class. Only a few families of the Fordhook backcross appear in the next class (40–59.9 percent). In other words, most of the families were relatively resistant to form 5, and there was no great difference between the reaction of F_3 or backcrosses to either parent. When tested with form 5 both parents and Sweetheart were in the lowest wilt class except in one case. This one exception was during the testing of the Russian backcrosses, when Fordhook and Sweetheart fell in the 40–59.9-percent class.

Form 8 was much more virulent, and when subjected to it more of the families showed a higher percentage of wilt. In fact nearly 10 percent of them fell in the 80–100-percent class. There was a larger percentage of the Russian than of the Early Fordhook backcrosses in the 0–19.9-percent class. This situation was reversed in the 20–39.9-percent class, while in the 40–59.9-percent class the Russian backcrosses again were in excess. In the two classes between 60 and 100 percent there was very little difference. The F_3 had the greater portion of its families in the low wilt class. The number in the progressively higher wilt classes gradually decreased without any distinct break. As with form 5, generally speaking, there was no great difference in reaction between the backcrosses to the two parents.

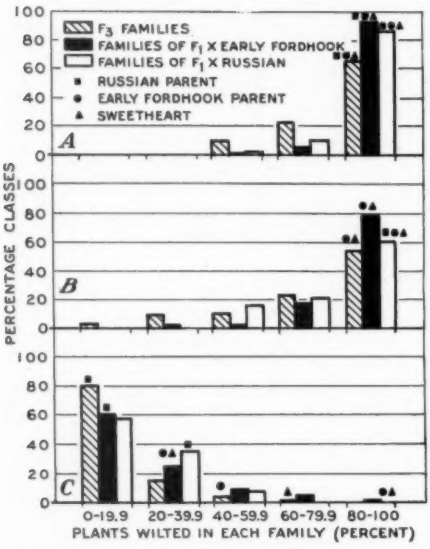

FIGURE 6.—Histograms showing the distribution of the families with respect to the total percentage of wilted plants per family during the tests with three forms of *Fusarium niveum*: *A*, form 20; *B*, form 8; *C*, form 5.

With form 20 the larger portion of the Russian backcross families was in the low wilt classes, with none in the 100-percent class, while the Fordhook backcross families were fairly well represented in all classes. There was a considerably smaller proportion of the Fordhook backcrosses in the low wilt classes than there was of the Russian backcrosses, showing very definitely the influence of the resistance of the backcross parent. The F_3 had a little over 30 percent of its families in the 0–19.9-percent wilt class, increasing to about 36 percent in the 20–39.9-percent class, then decreasing to 5 percent in the 80–100-percent class.

With forms 8 and 20 the Russian fell in lower wilt classes than did the Early Fordhook parent.

In order to determine the distribution and amount of wilt throughout the entire period, the tests were continued until most of the plants had wilted, and the number of plants remaining at the end of the test was recorded. In figure 6 is shown the proportion of families which fell in the various wilt classes based on the amount of wilt during the entire test. With form 5 the results are difficult to explain. The F_3 and Early Fordhook backcrosses had a larger proportion of families in the low wilt classes than did the Russian backcrosses, even though the Russian parent in all three cases showed a smaller amount of wilt than the Early Fordhook parent.

With forms 8 and 20 a larger proportion of the Russian than of the Fordhook backcross families were in the lower wilt classes, but an even greater proportion of the F_3 were in these classes.

With form 8 the Russian parent was in the 60–79.9-percent class, while the Early Fordhook was in the 80–100-percent class. With form 20 both parents were in the 80–100-percent class.

EVIDENCE OF TRANSGRESSIVE SEGREGATION

There were several families that showed greater resistance than the Russian parent and several that showed greater susceptibility than the Early Fordhook parent, indicating transgressive segregation.

CORRELATIONS

The correlations between the reactions to the three Fusarium forms were not high, as will be seen in table 1, but were generally higher at the end of the 4-day period than at the end of the test. There was a higher correlation between the reaction to forms 8 and 20 than there was between 5 and either 8 or 20. At the end of 4 days the correlation between the reaction to form 8 and the reaction to form 20 of the Russian backcross families is 0.72, while that between 5 and 20 is 0.48. According to Fisher's method (*2*) for testing the significance of differences between correlations, the difference in the z value for the two correlations is 0.45 ± 0.18. This is more than twice its standard error, indicating that the difference is significant. The correlation between the reaction to forms 8 and 20 of the F_3 families is greater than that between 5 and either 8 or 20 at the end of the 4-day period. Fisher's test shows that these differences are not significant.

INHERITANCE OF CERTAIN CHARACTERS

Notes were taken on certain characteristics of the plants as they were growing in the field and on the fruits at the time the seeds were removed. In the F_1 of the Russian × Early Fordhook and reciprocal crosses all of the Russian characters except fruit shape appeared to be dominant. The fruits borne on the F_1 plants were oval, as were those of the Early Fordhook parent. There appeared to be no simple segregation of the characters of the plants, although certain of the fruit characters suggested more definite ratios.

TABLE 1.—*Correlations between the action of the three Fusarium forms on the different groups of families* [1]

When correlated	Forms	Backcross to Early Fordhook (118 families)	F_3 (102 families)	Backcross to Russian (62 families)
At end of 4 days	5 and 8	0.14	0.54	0.46
	5 and 20	.15	.52	.48
	8 and 20	.32	.68	.72
At end of test	5 and 8	.21	.41	.02
	5 and 20	.04	.46	.09
	8 and 20	.53	.39	.45

[1] According to Fisher's V. A. table an r value of at least 0.1946 is necessary where $n=100$ for odds of 19 to 1 ($P=0.05$) for significance. For the same odds an r value of 0.2500 is necessary where $n=60$.

There was considerable variation in the color of the flesh, and those fruits classified as red ranged from a slight pink to a bright red. The results from the backcrosses to Early Fordhook suggest a single factor

for flesh color, but in the F_2 there was a large excess of white-fleshed fruits over the expected 3:1, only 2 red-fleshed ones out of 79 being obtained. The backcross to the Russian gave all white-fleshed fruits.

The data on texture of the flesh do not suggest any definite factorial interpretation. The seed-coat color of the Russian is green, while that of Early Fordhook is white, or nearly so. The F_1 was green. Many different colors, including brown, red, gray, and some gray with black margins in addition to the parental types, were obtained in the F_2 and back crosses.

DISCUSSION

Since homozygous inbred lines of the parents used in these studies were not established before the original cross was made, the results are probably not so clear-cut as they might have been. The amount of wilt undoubtedly was affected to a considerable extent by temperature and moisture fluctuations, as it was impossible to maintain a constant environment throughout the tests. The soil possibly became more heavily infested with the fungus as the testing proceeded, which probably would cause a more severe elimination in the later tests. One of the main difficulties in investigating the inheritance of resistance to many diseases has been that of repeatedly obtaining the same degree of infection on the same genotype. At the conclusion of several of the tests platings were made of apparently healthy plants. In most cases the fungus was recovered, indicating that resistance to wilt is due not necessarily to the failure of the fungus to enter the host but to its inability to develop sufficiently within the resistant plant.

In a cross of resistant×susceptible watermelon, Porter (*6*) obtained an F_1 that was as susceptible as the susceptible parent. The F_1 in the present work was intermediate between the parents but somewhat nearer the susceptible one.

In the study reported here, several factors for resistance appeared to be segregating in the F_2 and backcrosses since there were no definite breaks in the distribution of the families with regard to percentage of wilt. There was variability during the tests in the amount of wilt occurring in the parents, but of an average is taken the Russian shows less wilt than Early Fordhook, while Sweetheart shows more.

Since there is evidence of several factors governing resistance, and of transgressive segregation, it should be possible to isolate homozygous lines more resistant than the resistant parent, as well as many intermediate types; work done by the writer indicates that there is no close linkage between resistance and any of the other characters studied, and if this is true it should be possible to combine resistance and quality in the same melon. However, the difficulty in attaining this result is that both resistance and the other characters appear to be governed by several factors, making it necessary to select from a large population to get the desired combination of factors. The method that is being used at this station to obtain this result is to backcross the hybrids to the edible parent which is wilt-susceptible, then to self-pollinate the progeny in an effort to concentrate the genes for

resistance. Seeds from the more edible fruits are tested in the greenhouse for resistance. Then remnant seeds from those which proved most resistant are planted for backcrossing again, and the process is repeated. When the desired quality is obtained it will be necessary to continue selfing and selecting for the resistant genes for several generations until they too become homozygous. Thus far several melons have been obtained with considerable quality and a fair amount of resistance.

Possibly a shorter method than the one described would be to subject the backcross plants to a wilt test, and to transplant the survivors in the field, where they would be backcrossed again to the edible parent. A repetition of this process until the edible factors are fixed, then selfing with continued elimination of susceptible plants to render resistance homozygous, should give the desired result in a somewhat shorter time. The difficulties encountered with this plan have been to get the plants to live after transplanting, and even though they live they are often so stunted by the heavy infestation of wilt in the elimination test that they do not produce fruits.

SUMMARY

The object of this study was to determine how resistance to wilt in watermelons is inherited, and also to learn whether there is any correlation between the reaction to different forms of the wilt organism (*Fusarium niveum*).

Crosses were made between an inedible wilt-resistant watermelon introduced from Russia and Early Fordhook, a susceptible commercial variety. The chromosome number of the Russian variety was found to be 11 pairs, the same as that reported for the commercial varieties.

The F_3 and the progeny of selfed backcross plants were tested in the greenhouse with each of three physiologic forms of *Fusarium niveum*.

There was a greater proportion of families from backcrosses to the Russian parent in the resistant classes than from backcrosses to the Early Fordhook parent. Therefore it is concluded that resistance to wilt is inherited.

Since the F_3 and backcross families cannot be divided into distinct resistant and susceptible groups, resistance probably is dependent on several factors. At least a part of these seem to be cumulative in their action, since the reaction of the F_1 is intermediate between that of the parents.

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THE TOXICITY OF CERTAIN CHEMICAL AGENTS TO ERWINIA AMYLOVORA[1]

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INTRODUCTION

Extensive toxicological studies have been made with fungi, both pathogenic and nonpathogenic, and much progress has been made in developing fungicides that are highly efficient in combating many plant diseases. However, much less attention has been given to similar studies with bacterial pathogens of plants. Considerable progress has been made in seed-stock treatments, soil treatments, and local disinfection of woody plant parts, tools, etc., to combat bacterial plant diseases; and limited advances have been made in spraying and dusting against these maladies, chiefly with materials developed as fungicides. Nevertheless, whether because of the difficulties inherent in the problem or because of the need for further investigation, plant pathologists still lack spraying or dusting methods for bacterial diseases that compare in efficiency with those now in common use against fungus diseases of plants.

Brooks (*4*)[2], Miller (*17*), Shaw (*24*), and Keitt, Pinckard, and Riker, as reported by Christensen (*6, pp. 86–88*), have made a series of studies bearing on the epidemiology and control of fire blight of apple (*Malus sylvestris* Mill.), caused by *Erwinia amylovora* (Burr.) Bergey et al., in Wisconsin. Under the conditions thus far encountered in this State, water-borne bacteria from nearby lesions have seemed to comprise a major inoculum for both primary and secondary natural infection. Furthermore, it has been shown (*17*) that, under favorable conditions, the bacteria can infect young leaves, blossom buds, or blossoms by way of the stomata, without the intervention of wounds. While it is realized that major factors in the epidemiology of fire blight may vary greatly with regional and seasonal conditions, the type of epidemiological evidence encountered in Wisconsin suggested that increased attention be given to the development of bactericidal spray or dust treatments to aid in the control of this disease. Accordingly, in 1929, work was initiated along two lines: (1) Laboratory studies of the toxicity of various chemical compounds and spray materials to the fire blight organism, and (2) orchard spraying experiments. Progress reports have been made on the earlier results (*6, 11*). An account of the laboratory work on toxicity follows.

The work here reported was undertaken with two major aims: (1) To extend to a bacterial plant pathogen the type of toxicological study that has been applied to bacterial pathogens of man or animals, and (2) to study the toxicity to *Erwinia amylovora* of various types of chemical compounds and spray preparations in the hope of gaining

[1] Received for publication Feb. 10, 1936; issued October 1936. This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] Reference is made by number (italic) to Litrature Cited, p. 316.

information which would be of value in developing a bactericide for combating fire blight or other bacterial diseases of plants.

METHODS AND MATERIALS

Methods now available for studying the toxicity of chemical agents to bacteria have been developed chiefly in connection with problems of disease in man or animals. Major steps in the evolution of these methods may be traced in the works of Koch (*13*), Krönig and Paul (*14*), Rideal and Walker (*23*), the Lancet Commission (*1*), Anderson and McClintic (*2*), and Reddish (*19*). Reviews of the literature under discussion have been given in the Lancet (*1*) and by Rideal and Rideal (*22*) and Reddish (*20*).

Several investigators have studied the toxicity of various chemical agents to bacteria pathogenic on plants. Jehle (*9*) tested the toxicity of various disinfectants to *Phytomonas citri* (Hasse) Bergey et al., using a modification of Hill's method, a glass-rod method, a platinum-loop method, and a filter-paper method. Lee (*15*) studied the action of certain fungicides on the same organism, basing his technique on that of Anderson and McClintic (*2*). McCown (*16*) studied the toxicity of bordeaux mixtures and certain other materials to *Erwinia amylovora*. Suspensions of the bacteria were placed (1) in the toxic suspensions in test tubes or (2) on dry spray or dust residues on glass slides in Petri plates. At stated intervals a loop transfer was made from the medication tube or slide to a tube of an agar medium, which was then plated and incubated. The colonies that developed in the plates were counted. Controls were run without medication. Miller (*17*) tested the toxicity of several fungicidal preparations to *E. amylovora*, using a modification of the method of Anderson and McClintic. Thomas (*25*) has recently reported a method for phenol coefficient studies for use with bacterial pathogens of plants, based on the Rideal-Walker (*23*), Anderson and McClintic (*2*), and Reddish (*19*) techniques.

An account of the method used by the present writers, which is based mainly upon the techniques of Rideal and Walker (*23*) and Anderson and McClintic (*2*), as modified by Reddish (*19*), follows.

PREPARATION OF THE CULTURES

The medium was composed of peptone, 10 g; beef extract, 3 g; dextrose, 10 g; and distilled water, 1,000 cc; and was adjusted between pH 6.9 and 7.0 with potassium monohydrogen phosphate ($K_2HPO_4.3H_2O$). This broth was dispensed in 10-cc quantities in test tubes of uniform bore, which were then plugged with cotton and autoclaved for 20 minutes at 15 pounds pressure. The reaction was again determined, and readjusted in the unusual cases when this was necessary. The medium was incubated several days at room temperature before use.

Transfers of the cultures employed were made on at least 3 consecutive days immediately preceding medication.

The transfer needles were made of no. 23 U. S. standard gage chromel wire, with a 4-mm loop which would lift an unbroken film.

The incubation temperature, 24° C., was selected because it is near the optimum for growth of the bacteria and is favorable for development of fire blight.

The incubation period, 20 hours, was chosen with the aim of using the culture shortly prior to the development of the maximal percentage of living cells. Buchanan (*5*) has shown that the largest percentage of viable bacterial cells in culture occurs during the logarithmic growth phase. Counts made according to the method of Breed and Brew (*3*) on two broth cultures after incubation periods of 10, 16, 24, and 34 hours, respectively, at 24° C. showed a rapid increase in the number of cells up to the 24-hour period. The fact that single-cell isolations from 20-hour-old cultures were very successful is added evidence that a high percentage of cells in such cultures are viable.

PREPARATION AND STANDARDIZATION OF THE BACTERIAL SUSPENSIONS

A 20-hour-old broth culture of *Erwinia amylovora* prepared as described above was centrifuged for 25 minutes at approximately 2,500 revolutions per minute. The supernatant liquid was decanted, and 10 cc of an aqueous 0.85-percent solution of sodium chloride was added to the residue. The bacterial cells were suspended in the salt solution by agitation and this suspension was centrifuged as before. The supernatant liquid was decanted and sterile distilled water was added to the residue, with agitation, until the turbidity of the bacterial suspension matched that of a barium sulphate suspension which was prepared by adding 1.0 cc of a 1.0-percent solution of barium chloride ($BaCl_2.2H_2O$) to 99 cc of a 1.0-percent solution of sulphuric acid. This dilution usually required from 9 to 12 cc of water. The bacterial suspension, which was then ready for exposure to the toxic agent, contained approximately 110,000,000 cells per cubic centimeter, as was determined by direct counts of stained cells in a Levy counting chamber. The methods used in washing the bacteria were adapted from the technique of Klarmann, Shternov, and Von Wowern (*12*). The time that the bacteria remained in distilled water varied from a few minutes to 3 hours, depending on the number of series of tests (1 to 4) for which each suspension was used. Winslow and Brooke (*27*) have shown that certain bacteria are killed when centrifuged and suspended in distilled water, while others are relatively resistant to such treatment. In the present work results from the experiments in which a single bacterial suspension was used for four successive series through a 3-hour period were as consistent as those in which the bacteria were used after being suspended only a few minutes in water.

PREPARATION OF THE TEST SOLUTIONS

Lipped pyrex test tubes 150 by 16 mm (over all) were used in the earlier work. Later pyrex tubes 100 by 25 mm were substituted to facilitate manipulations of seeding and transfer without touching the walls of the tube and to expedite uniform suspension of the test organism by quick agitation. All glassware used in preparing the test solutions and conducting the test was chemically cleaned, sterile, and dry.

Unless otherwise noted, the test solutions were prepared as follows: A suitable quantity of the test material[3] was dissolved in sterile distilled water. If possible, concentrations were expressed in terms of

[3] The test materials were not sterilized. Contaminations were very rare, the few that occurred being chiefly in materials of which the strongest obtainable concentrations were of low toxicity (e. g., lime-sulphur and calcium carbonate).

molar solutions; if not, as in the case of spray materials, in terms of grams per cubic centimeter or pounds per gallon in water. In the case of molar solutions, 10 dilutions of the test solution were made in geometrically decreasing concentrations. A portion of the original solution was diluted to the highest of the desired concentrations, and 5 cc placed in a tube. Five cubic centimeters of this same concentration was then added to a tube containing 5 cc of sterile distilled water. This solution (10 cc) was thoroughly agitated, and 5 cc removed to another tube containing 5 cc of water. This procedure was continued until the 10 dilutions were obtained.

Before the test organism was introduced, the test solutions were examined for sterility by transferring a loop from each medication tube to a sterile tube of the culture medium. These control tubes were incubated with the other transfers mentioned below. On the rare occasion when growth appeared in a control tube, the results from this dilution were discarded.[3] If the contaminated tube was of a dilution that entered into the significant results of the series, the entire series was discarded.

THE TEST

Two test-tube racks with a capacity of 10 tubes each were immersed in a water bath similar to that described by Anderson and McClintic (*2*). The bath was held at 20° C. ± ½°. During the experiment, tubes containing the test organism and test solutions, the latter arranged from left to right in order of decreasing concentration, were immersed below the level of the liquid in each tube. After the experimental materials were in thermal equilibrium with the bath, the bacterial suspension was agitated and 0.5 cc was placed in the highest concentration of the test solution. Care was taken to place the test organism in the test solution rather than on the side of the tube. The tube was then gently shaken. At intervals of 30 seconds, the lower concentrations were similarly treated in succession. The last tube received the test organism 4½ minutes after the first. A transfer to a tube of the nutrient dextrose broth described above was made from each medication tube 5 minutes after the bacteria were introduced. Care was taken to dip the transfer loop to the bottom of the medication tube and withdraw it in a standardized manner without touching the sides or losing the adhering drop of liquid. A similar series of transfers was made after the bacteria had remained for 10 minutes in the medication tubes. The subculture tubes and suitable controls were incubated for 48 hours at 24° before results were recorded. The presence or absence of growth in the subculture tubes was taken as an index of toxicity.

The addition of 0.5 cc of the bacterial suspension to the test solution, in accordance with the widely used method of Reddish (*19*), causes a noteworthy dilution of the toxic solutions. If desired, this can readily be corrected in making the dilutions.

SOURCE AND PURITY OF THE CULTURES

One culture (or isolate) was used for most of the work. This was derived from a single bacterial cell isolated by the method of Wright et al. (*28*). The original culture came from a pear canker which was obtained from Arkansas. However, in view of the wide variability

commonly exhibited by micro-organisms, it seemed undesirable to limit the study to one culture. Consequently, isolates from widely separated sources were compared with this standard culture in a smaller number of trials.

The cultures (table 1) are designated by letters indicating the States from which the material for isolations was obtained. Cultures purified by isolation of single cells (*28*) are indicated by capital letters, e. g., ARK.

During the progress of the work, the pathogenicity of the cultures was tested at approximately monthly intervals by inoculating them into young apple shoots grown in the greenhouse. All were continuously pathogenic.

TABLE 1.—*The toxicity of certain chemical agents to Erwinia amylovora*

Compounds tested	Cultures[1]	Total trials	Medication	Experiments in which there was no growth of the test organism following medication at a molecular concentration [2] of—										
				2M	M/1	M/2	M/2^2	M/2^3	M/2^4	M/2^5	M/2^6	M/2^7	M/2^8	M/2^9
		Number	*Minutes*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
$CuSO_4.5H_2O$	ARK	6	5	100	50	0	----	----	----	----	----	----	----	----
			10	100	83	33	17	0	----	----	----	----	----	----
$ZnCl_2$	do	6	5	100	0	----	----	----	----	----	----	----	----	----
			10	100	83	0	----	----	----	----	----	----	----	----
Do	Ark	2	5	100	0	----	----	----	----	----	----	----	----	----
			10	100	0	----	----	----	----	----	----	----	----	----
K_2CrO_4[3]	ARK	3	5	----	100	0	----	----	----	----	----	----	----	----
			10	----	100	67	0	----	----	----	----	----	----	----
$FeCl_3.6H_2O$	Ark, Ind, Oreg	3	5	----	----	----	67	0	----	----	----	----	----	----
			10	----	----	----	100	0	----	----	----	----	----	----
$NiCl_2.6H_2O$	Ark, Oreg	2	5	----	----	----	0	0	----	----	----	----	----	----
			10	----	----	----	100	50	0	----	----	----	----	----
Na_2CO_3[4]	ARK	2	5	----	----	----	100	50	0	----	----	----	----	----
			10	----	----	----	----	100	50	0	----	----	----	----
$CuCl_2.2H_2O$	do	6	5	----	----	----	----	100	17	17	0	----	----	----
			10	----	----	----	----	100	83	50	0	----	----	----
Do	Ark	2	5	----	----	100	50	50	50	0	----	----	----	----
			10	----	----	----	----	100	50	50	50	0	----	----
$CH_3.COOH$[5]	ARK	2	5	----	----	----	100	50	0	----	----	----	----	----
			10	----	----	----	----	100	0	----	----	----	----	----
$FeSO_4.7H_2O$	Ark	3	5	----	----	----	----	100	67	0	----	----	----	----
			10	----	----	----	----	100	67	0	----	----	----	----
K_2CO_3[3]	do	3	5	----	----	----	100	67	33	0	----	----	----	----
			10	----	----	----	----	----	100	33	33	0	----	----
$H_3AsO_4.\frac{1}{2}H_2O$	do	2	5	----	----	----	----	100	0	----	----	----	----	----
			10	----	----	----	----	----	100	0	----	----	----	----
$CdCl_2.2\frac{1}{2}H_2O$	do, Ind	2	5	----	----	----	----	----	100	50	50	0	----	----
			10	----	----	----	----	----	100	50	100	0	----	----
$Ca(OH)_2$[6]	ARK	3	5	----	----	----	----	----	----	100	0	----	----	----
			10	----	----	----	----	----	----	100	0	----	----	----
HCl	do	3	5	----	----	----	----	----	----	----	100	0	----	----
			10	----	----	----	----	----	----	----	----	100	0	----
Do.[4]	Ark	2	5	----	----	----	----	----	----	100	0	----	----	----
			10	----	----	----	----	----	----	100	50	0	----	----
$C_6H_5.OH$	do	2	5	----	----	----	----	----	----	100	0	----	----	----
			10	----	----	----	----	----	----	100	0	----	----	----
NaOH	ARK	5	5	----	----	----	----	----	----	100	40	20	0	----
			10	----	----	----	----	----	----	100	80	40	20	0
Do.[7]	Ark	5	5	----	----	----	----	----	----	100	40	0	----	----
			10	----	----	----	----	----	----	100	60	0	----	----
KOH[4]	do	3	5	----	----	----	----	----	----	100	33	0	----	----
			10	----	----	----	----	----	----	100	67	0	----	----
$Fe_2(SO_4)_3$[6]	do	2	5	----	----	----	----	----	----	----	100	50	0	----
			10	----	----	----	----	----	----	----	----	100	0	----

See footnotes at end of table.

TABLE 1.—*The toxicity of certain chemical agents to Erwinia amylovora*—Contd.

Compounds tested	Cultures	Total trials	Medication	Experiments in which there was no growth of the test organism following medication at a molecular concentration of—												
				M/2[9]	M/2[10]	M/2[11]	M/2[12]	M/2[13]	M/2[14]	M/2[15]	M/2[16]	M/2[17]	M/2[18]	M/2[19]	M/2[20]	M/2[21]
		No.	Min.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
$Hg(CN)_2$	ARK	7	5	100	86	0										
			10		100	72	14	0								
Do [6]	WIS	2	5		100	50	50	0								
			10			100	50	0								
C_2H_5HgCl [6]	Ark., WIS., WIS.	3	5					100	66	66	0					
			10					100	66	66	36	0				
$HgCl_2$	ARK	1	5									100	0			
			10								100	0				
Do	do	1	5									100	0			
			10									100	0			
Do	do	1	5									100	0			
			10										100	0		
Do	do	1	5									100	0			
			10										100	0		
Do	do	4	5										100	0		
			10										100	0		
Do	do	2	5										100	0		
			10										100	0		
Do	do	1	5										100	0		
			10											100	0	
Summary		11	5									100	60	27	0	
			10								100	91	82	68	9	0
$HgCl_2$	N. Y.	4	5									100	75	0		
			10								100	50	25	0		
Do	Ark	6	5								100	67	0			
			10									100	67	33	0	
Do	Wis	2	5								100	50	0			
			10									100	0			
Do	N. Y.	2	5									100	50	0		
			10										100	0		
Do	N. Y., Wis.	2	5									100	0			
			10										100	0		
Do	Ark	2	5									100	50	50	0	
			10										100	50	0	
Summary		29	5								100	90	41	14	0	
			10								100	93	79	35	3	0
HgO [6]	ARK	6	5								100	83	50	17	0	
			10									100	83	33	17	0
HgCl [6]	do	6	5									83	67	33	0	
			10									100	83	33	33	0
Ag_2SO_4	do [9]	2	5										100			
			10										100			

[1] Letters indicate State from which material for isolations was obtained, capital letters being used for cultures purified by isolation of single cells.

[2] For various reasons certain solutions were not used at the exact concentrations shown in the table. In these cases the stated concentrations should be corrected by multiplying by the factors given in footnotes 3 to 8, inclusive:

[3] 0.75; [4] 1.13; [5] 1.08; [6] 1.56; [7] 1.27; [8] 0.78.

[9] The test organism was washed in sterile distilled water, instead of physiological salt solution, to prevent precipitation of silver chloride.

SOURCE AND PURITY OF THE CHEMICAL AGENTS TESTED

The phenol used met the requirements of the Tenth United States Pharmacopoeia. Its congealing point was 39°–40° C.

The other chemical agents tested, except as stated below, were of the highest purity carried in stock by leading chemical supply houses, being of analytical reagent, C. P., or U. S. P. grade.

The calcium hydroxide was freshly prepared from calcium nitrate and ammonium oxalate of analytical reagent grade and tested for purity by the method of Treadwell and Hall (*26*).

The ethyl mercuric chloride was made from commercial chemicals of high purity by a manufacturer of fungicides.

The lime-sulphur was a high-grade commercial liquid concentrate of 32° B. at 24° C.

The bordeaux and zinc-lime mixtures were prepared from reagents of C. P. grade, except that the calcium caseinate used in the latter mixture was a standard commercial product commonly used in practice.

RESULTS FROM MOLAR SOLUTIONS

The results of the main body of the work appear in table 1, expressed in terms of the percentage of experiments in which there was no growth of the test organism following medication at the stated concentrations. To facilitate comparisons, the chemical agents tested are arranged in the table, as nearly as feasible, in the order of increasing toxicity.

An examination of the data indicates that as much variation occurred in the results from the single-cell isolate ARK as in those from the various other isolates used. Consequently, the data from the different isolates used in testing a given material are not segregated, except in the case of the work on mercuric chloride where, for comparison, they are given separately and in summary. It will be observed that the summarized data from isolate ARK are in very close agreement with the corresponding data from the various isolates.

Under the conditions of these tests, the following salts of heavy metals showed comparatively low to moderate toxicity to *Erwinia amylovora*, in increasing order: Cupric sulphate ($CuSO_4.5H_2O$), zinc chloride ($ZnCl_2$), ferric chloride ($FeCl_3.6H_2O$), nickel chloride ($NiCl_2.6H_2O$), cupric chloride ($CuCl_2.2H_2O$), ferrous sulphate ($FeSO_4.7H_2O$), cadmium chloride ($CdCl_2.2\frac{1}{2}H_2O$), and ferric sulphate ($Fe_2(SO_4)_3$). The greater toxicity of copper chloride than copper sulphate, and the reverse relations of ferric chloride and ferric sulphate, are of theoretical interest, and suggest the desirability of physiological and physicochemical studies which are beyond the scope of the present work.

Hydrochloric acid (HCl), as a representative mineral acid, and sodium and potassium hydroxides (NaOH and KOH), as representative hydroxides, showed about equal toxicity, approximating that of phenol ($C_6H_5.OH$). Acetic acid ($CH_3.COOH$) and orthoarsenic acid ($H_3AsO_4.\frac{1}{2}H_2O$) were distinctly less toxic.

Sodium carbonate (Na_2CO_3) and potassium carbonate (K_2CO_3) each approximately equaled acetic acid in toxicity.

Potassium chromate (K_2CrO_4), under the conditions of these experiments, was one of the least toxic materials tested.

The mercury and silver compounds were by far the most toxic materials studied. Mercuric cyanide ($Hg(CN)_2$) was notably the least toxic of the mercury compounds tested, ethyl mercuric chloride (C_2H_5HgCl) was intermediate, and mercuric chloride ($HgCl_2$), mercurous chloride (HgCl), and mercuric oxide (HgO) were the most toxic.

Silver sulphate (Ag_2SO_4), in two experiments, was slightly more toxic than any of the mercury compounds, being lethal at the greatest dilution tested, $\frac{M}{2^{18}}$.[4]

[4] See footnote 9 of table 1.

RESULTS FROM SPRAY MATERIALS

In addition to the work with molar solutions, tests were made of certain spray materials. The same technique was employed, except that dilutions were based on spray formulae, or, in the case of lime-sulphur, dilution by volume with water. The results will not be given in detail. The following excerpts are from the data from tests with the 10-minute medication period:

Bordeaux mixture.—In 15 experiments the formula of 0.5 pound $CuSO_4.5H_2O$, 1.5 pounds CaO, and water to make 50 gallons was always lethal; one-half of this concentration was never lethal.

Lime-sulphur (commercial liquid concentrate, 32° B.).—In six experiments at full strength it was always lethal. When diluted one-half with water, it was lethal in 50 percent of the tests. In no case was it lethal at 1 gallon in 20.

Zinc sulphate-lime spray.—In four experiments the formula of 2 pounds $ZnSO_4.7H_2O$, 1.5 pounds CaO, 0.75 pound of a commercial calcium caseinate spray supplement, and water to make 50 gallons was always lethal. A formula three-fourths as strong was lethal in 75 percent of the tests; one-half as strong, in 25 percent.

DISCUSSION

It is generally accepted that the toxicity of chemical agents to micro-organisms varies greatly with environmental conditions. This fact contributes much to the difficulty of making exact comparisons of the relative toxicity of a chemical agent to different test organisms, especially when the latter differ widely in their environmental requirements. In the present work substantial modifications of the basic methods (*1*, *19*, *23*), which were devised for use with bacteria pathogenic on man, were necessary to adapt the technique to a test organism pathogenic on plants. Consequently, the results are not strictly comparable with those which have been obtained by the Rideal-Walker method and its modifications on the germicidal efficiency of chemical agents against bacteria that cause disease in man. It has, therefore, seemed unnecessary to follow the Rideal-Walker and similar methods in expressing results in terms of phenol coefficients. Since the major purpose of the present work was to compare the toxic effects of various chemical agents to a single test organism under conditions suited to its environmental requirements, it has seemed more useful to express the results in terms of presence or absence of growth after given medication periods at stated concentrations of the test materials, using molar solutions if feasible and showing for each concentration the percent of the tests in which no growth occurred. The use of solutions based on molecular concentration has important advantages that have long been recognized in work on the toxic effects of chemical agents on fungi (*6*, *7*, *29*), seed plants (*10*), and bacteria (*18*).

Thomas (*25*) computed for each time period of medication used in his work the ratio of the lowest concentration of the test material that stopped growth to the lowest concentration of phenol that stopped growth, and took the average of these as the phenol coefficient. Expression of such results in terms of coefficients that relate them to a standard appears to be useful if the major purpose is, as in the work of Thomas, to compare the relative germicidal efficiency of various chemical agents to a group of organisms. Obviously, however, such coefficients are strictly comparable only with others determined by the same technique.

The data in table 1 show that for most compounds tested the lowest concentration which prevented growth varied by one to several dilutions in successive experiments with the same material. Such variations in responses of bacteria to techniques of the type here employed are to be expected, and are in accord with the results obtained by Esty and Williams (*8*) in studies of thermal death times. This variability adds to the difficulty of accurate expression of results of toxicological studies in terms of coefficients.

Cognizance should be taken of the fact that such laboratory studies as are here reported are necessarily conducted under highly artificial conditions, and do not take into account some of the most important factors that determine the bactericidal efficiency of a material for use in the field, e. g., its resistance to removal by meteoric water. Furthermore, the time periods of medication, 5 and 10 minutes, were chosen primarily because of their extensive use in toxicological studies, rather than as paralleling natural conditions. The results, therefore, bear on the potentialities of materials as field bactericides only insofar as they reveal their comparative toxicity under the experimental conditions.

The data in table 1 show that, under the conditions of these experiments, cupric sulphate and zinc chloride, both of which are used in formulae employed in fire-blight control, are among the least toxic of the chemicals tested.

All the mercury compounds tested were very highly toxic to *Erwinia amylovora*, mercuric cyanide and ethyl mercuric chloride being, however, distinctly less toxic than mercuric chloride, mercurous chloride, and mercuric oxide.

Thomas (*25*) found mercuric chloride and ethyl mercuric chloride to be highly toxic to *Erwinia amylovora*. Mercuric chloride and mercuric cyanide have long been used as valuable disinfecting agents for treatment of wounds and tools in the excision of diseased parts of plants affected by fire blight (*21*).

The high toxicity of its compounds to *Erwinia amylovora* seems to make mercury an attractive material for further study in relation to its possible adaptations for increased use in fire-blight control.

Of the orchard sprays tested, lime-sulphur was notable for its low toxicity to the fire blight organism. Field experiments have supplemented the laboratory data in indicating that this fungicide, as commonly used in apple spraying, has little or no value against fire blight. The fire blight organism was easily and repeatedly isolated by plating from spray residues from uninfected leaves on severely blighted trees which had been sprayed 1 to 2 days previously with lime-sulphur, 1 gallon in 50, and lead arsenate, 1 pound in 50 gallons of spray. Lime-sulphur is widely used in often-repeated applications against fungus diseases of apple during a period when a spray suitably toxic to *Erwinia amylovora* might be of substantial value in fire-blight control.

Bordeaux mixture and the zinc sulphate-lime spray were lethal to the fire-blight bacteria at less than the concentrations commonly recommended for orchard use. Lee (*15*) concluded that bordeaux is valueless against *Phytomonas citri*, unless lime is used in excess, and apparently attributes the toxicity of bordeaux to this organism to the calcium hydroxide in solution. McCown (*16*) reported that, in his laboratory tests, "neutral" bordeaux was almost as effective as alka-

line bordeaux, and concluded that, therefore, some other factor in addition to the hydroxyl ion is necessary to account for the toxic effects of these sprays on *Erwinia amylovora*. An analysis of the extent to which the bactericidal action of bordeaux and similar mixtures is attributable to the excess of lime, as compared with other constituents of these preparations, lies beyond the scope of the present paper.

SUMMARY

The toxicity of various chemical compounds and spray preparations to *Erwinia amylovora* was studied by a method based chiefly on the techniques of Rideal and Walker and Anderson and McClintic, as modified by Reddish.

Most of the work was done with cultures derived from the progeny of a single cell. As much variation occurred in the results from this single-cell isolate as from various other isolates which were studied comparatively.

The comparative toxicities of the several materials tested are recorded and discussed.

The high toxicity of the mercury compounds tested suggests the desirability of further studies concerning the possible adaptation of mercury for increased use in fire-blight control.

Lime-sulphur, which is widely used against fungus diseases of apple and pear during a period when a spray suitably toxic to *Erwinia amylovora* might be of substantial value in fire blight control, showed little or no toxicity to the blight organism at the concentrations used in practice.

Bordeaux mixture and the zinc sulphate-lime spray were lethal to the fire-blight bacteria at less than the concentrations usually recommended for orchard use.

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U. S GOVERNMENT PRINTING OFFICE: 1936